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SOIL SCIENCE

Contents for May, 1921

HYDROGEN-ION CONCENTRATION RELATIONS IN A THREE-SALT SOLUTION

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INTRODUCTION

The interest of a considerable number of workers, using water-cultures, in solving various phases of the salt-requirements of plants or allied problems, has centered itself in part on attempting to find a desirable control solution. Such a solution should be as simple as possible and defined in such a way as to be duplicated readily by other workers. It would be defined physiologically, in terms of the plant's response; chemically, by a statement of the molecular proportions of its component salts; and physically, by the magnitude of its osmotic properties. A still more complete statement would include the degree of true acidity or alkalinity existing in the solution, as determined by the hydrogen-ion concentration.

Knop's (8), Tottingham's (18), and Shive's (14) 3-salt solutions, have all been recognized as standard solutions. Little is known, however, concerning their true reaction. It was considered worth while to repeat Shive's work with the purpose of determining the part played by the hydrogen-ion concentration in the 36 different sets of salt proportions which he employed.

The publication by the Special Committee on Salt Requirements of Representative Agricultural Plants (16) resulted in our modifying our original purpose to the extent of using instead of the 36 sets of salt proportions having an osmotic concentration of 1.75 atmospheres, the 21 sets of salt proportions of type I with an osmotic concentration of 1.00 atmosphere.

The importance of the true reaction of a culture medium as affecting its biological activities is well recognized. However, only relatively few studies have been made of the direct physiological influence of reaction as measured by the hydrogen-ion concentration upon plants grown in solution-culture.

Toole and Tottingham (17) found a correlation between the weight yield of tops and the hydrogen-ion concentration of the solution—"these two values varying in opposite directions." Hoagland (5) carried out solution-culture experiments in which the concentration, composition and reaction were under control. Intensity of reaction was determined by means of the H-electrode. Salter and McIlvaine (12), more recently, have determined the effect of reaction upon the growth and germination of seeds of wheat, soybean,

corn and alfalfa. A reaction of 2.16 pH was fatal to the seedlings of all the plants, while a reaction of 7.71 pH depressed the growth of all except the corn seedlings.

MATERIALS AND METHODS

The methods used in the preparation of the culture solutions, the germination of the seed and the treatment of the plants throughout the growth period, were those recommended by the Committee on Salt Requirements of Repre-

TABLE 1

Partial volume-molecular concentrations and molecular proportions of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in the 21 solutions, differing by increments of one-eighth in the salt proportions, but all having an osmotic value of approximately 1.00 atmosphere at 25°C.

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
IR_1S_1	1	1	6	0.0027	0.0027	0.0161
S_2	1	2	5	0.0025	0.0049	0.0123
S_3	1	3	4	0.0024	0.0071	0.0094
S_4	1	4	3	0.0022	0.0089	0.0067
S_5	1	5	2	0.0022	0.0108	0.0043
S_6	1	6	1	0.0020	0.0122	0.0020
R_2S_1	2	1	5	0.0053	0.0027	0.0132
S_2	2	2	4	0.0049	0.0049	0.0099
S_3	2	3	3	0.0047	0.0071	0.0071
S_4	2	4	2	0.0045	0.0090	0.0045
S_5	2	5	1	0.0041	0.0104	0.0021
R_3S_1	3	1	4	0.0076	0.0025	0.0101
S_2	3	2	3	0.0072	0.0048	0.0072
S_3	3	3	2	0.0068	0.0068	0.0045
S_4	3	4	1	0.0065	0.0086	0.0021
R_4S_1	4	1	3	0.0099	0.0025	0.0074
S_2	4	2	2	0.0094	0.0047	0.0047
S_3	4	3	1	0.0090	0.0068	0.0022
R_5S_1	5	1	2	0.0123	0.0024	0.0049
S_2	5	2	1	0.0118	0.0047	0.0023
R_6S_1	6	1	1	0.0145	0.0024	0.0024
Shive's	3.77	1.09	3.14	0.0180	0.0052	0.0150
K*				0.0044	0.0145	0.0050
T†				0.0108	0.0101	0.0081

(In addition to the three salts listed above, Knop's, K, and Tottingham's, T, solutions also contain potassium nitrate in the concentrations as given.)

* K — KNO_3 = 0.0059.

† T — KNO_3 = 0.0034.

sentative Agricultural Plants (16). Table 1 gives the molecular proportions and the volume-molecular concentration of the solutions used. The relation of the 21 solutions of type I to each other may be shown readily by the use of the triangle diagram (fig. 1) described in various publications.[1] In addition to the 21 sets of salt proportions varying in increments of one-eighth of the total volume-molecular concentration, a single culture in distilled water was conducted during each of the three time-periods as well as triplicate cultures of Shive's "best," R_5C_2, having an osmotic concentration of 1.75

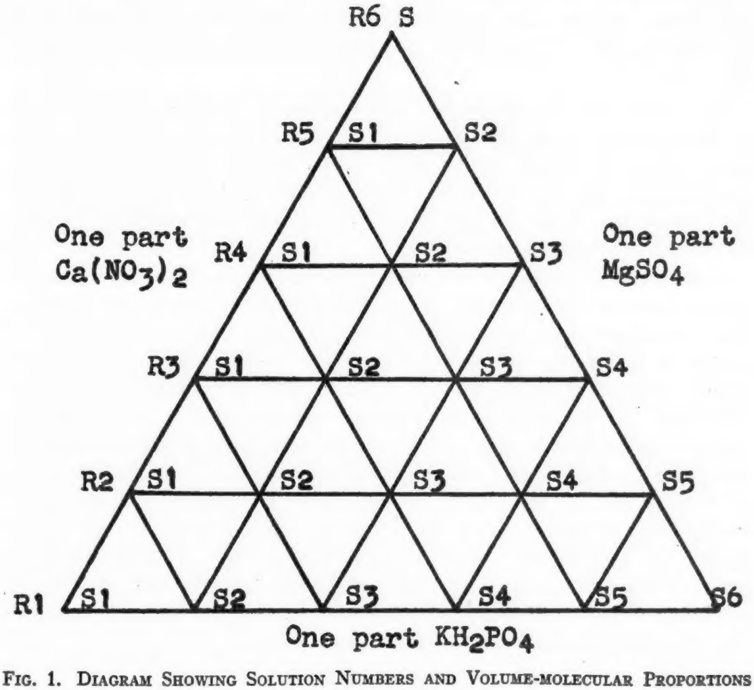

FIG. 1. DIAGRAM SHOWING SOLUTION NUMBERS AND VOLUME-MOLECULAR PROPORTIONS OF THE THREE SALTS

atmospheres. For purposes of comparison, there were conducted during the latter two time-periods, duplicate cultures of Knop's solution (designated as K in all of the tables) and Tottingham's best solution (designated as T in all of the tables), each having an osmotic concentration of 1.75 atmospheres (14).

The wheat plants used in this series of experiments belonged to the Fulcaster variety. The seed was obtained from the United States Department of Agriculture. The method of germination used was that described by the Plan of the Committee on Salt Requirements, etc. (16). Seedlings of uniform

[1] For the application of the triangle diagram to studies in plant nutrition, see Schreiner and Skinner (13), Tottingham (18), Shive (14) and Johnson (7).

height were selected and threaded through holes in paraffined corks, prepared as suggested by Tottingham (18). Each culture bottle contained 6 seedlings. The jars used to contain the culture solutions were of white flint glass similar in dimensions to those used by Shive, but having a capacity of slightly more than 210 cc. Before being used, the jars and all the glassware used in the experiments was cleansed with chromic acid cleaning fluid, steamed in the autoclave, treated with alkali, followed with cleaning fluid, rinsed several times with tap water and finally four times with distilled water.

To protect the roots in the culture jars from the light and excessive absorption of heat, the jars were wrapped in paper jackets which were black inside and white outside. These jackets or wrappers were prepared as described by Shive (14).

The solutions in the culture jars were replaced at the end of each $3\frac{1}{2}$ days by fresh solutions of similar volume. Hence the solutions were renewed nine times during the growth period of 35 days. As the rate of absorption is approximately the same as the rate of transpiration in wheat (10), we have in the amount of solution absorbed a measure of the transpiration; these data are recorded elsewhere as transpiration.

Since it was impossible to control the external conditions under which the cultures were to be conducted, the alternative was to submit all the plants to these conditions in an approximately similar way. This was accomplished practically by placing the cultures on a slowly rotating table; the cultures in a given circle on the table were subjected to the same variations in the surroundings. As the solutions were always used in duplicate there were two rows of circles on the table. The outside row is designated throughout this paper as series A, and the inner row, as series B. Thus the two circles or series of cultures for a given growth period had slightly different aerial surroundings, although the different cultures of the same circle were comparable. The rotating table used in this work was 4 feet in diameter and was attached to a ball-bearing base. The table was rotated by a small $\frac{1}{12}$-horsepower motor, belted to a reducing gear, which, in turn, was belted to the table. The table made a complete revolution every 4 minutes.

As it was appreciated that seasonal variations influence the physiological condition of the plant and hence the growth rate—the culture series were conducted in duplicate through three different time-periods of the year. It was hoped by this means to eliminate seasonal variation and to arrive, at least approximately, at the physiological properties of the solutions as determined by the chemicals alone. The first duplicate series was conducted from July 1 to August 5, 1919. The second duplicate series was conducted from November 23 to December 28, 1919; and the third from January 21 to February 25, 1920. The length of each time-period was 35 days. The period from July 1 to August 5 is characterized throughout this paper as the "first time-period," the period from November 23 to December 28, as the "second time-period;" and the period from January 21 to February 25 as the "third time-period."

Determination of hydrogen-ion concentration

The initial reaction of the culture solutions was determined and also the reaction at the end of each 3½-day interval, when the solutions were renewed. The hydrogen-ion concentrations of the solutions employed in the experiments recorded here were determined colorimetrically according to the method of Clark and Lubs (1). The recommendations of these authors in the purification of the salts for the buffer mixtures, were followed carefully. Although it is desirable to check the values of the buffer mixtures against the hydrogen electrode, it is not altogether necessary when relative rather than absolute values are desired. Clark and Lubs have pointed out that the absolute accuracy of the buffer mixtures is far within the experimental error that occurs in determining differences of color value between an unknown and the standards. Gillespie and Hurst (3, 4), using buffer mixtures prepared similarly in their determinations on soils, checked the mixtures electrometrically. They confirm the values assigned by Clark and Lubs and the accuracy of the composition of the buffer mixtures, adding that "if the substances used are pure this is unnecessary."

RESULTS

Aerial conditions

Records were obtained for: (a) air temperature; (b) relative humidity; (c) evaporating power of the air; (d) intensity of the absorbed radiant energy; and (e) duration of sunshine.

(a) Temperature changes and changes in the relative humidity were recorded on a hygro-thermograph placed near the rotating table. The maximum and minimum changes of temperature were recorded daily from the thermograph sheet and the mean temperature calculated. The data of these changes are expressed in terms of the Centigrade scale.

(b) The changes in the relative moisture-holding capacity of the air were recorded daily, and show maximum, minimum, and the mean expressed as relative humidity. The hygrograph was standardized at the beginning of each time-period by means of a sling psychrometer and the relative humidity determined by reference to Marvin's (11) psychrometric tables.

(c) The evaporating power of the air was measured by means of white and black standardized spherical porous cup atmometers [Livingston (9)]. The atmometers were weighed and cleaned weekly. Readings were corrected by multiplying by the coefficient of correction of the cup used.

(d) The intensity of the absorbed radiant energy [Livingston (9)] is expressed as the difference in the losses between the black cup and the white cup. Both white and black cups were placed on the innermost circle on the rotating table along with one of the control solutions and the culture in distilled water.

(e) The duration of sunshine expressed in hours, was obtained from the records of the Marvin Sunshine Recorder of the United States Weather Bureau Station located about 300 yards from the greenhouse in which these experiments were performed.

A summary of the aerial conditions for each time-period is given in table 2.

TABLE 2

Weekly averages of aerial conditions for the three time-periods

DATE, WEEK ENDING	ATMOMETRIC DATA (CORRECTED)			SUNSHINE*	RELATIVE HUMIDITY			TEMPERATURE		
	Loss white cup	Loss black cup	Difference		Average maximum	Average minimum	Mean	Average maximum	Average minimum	Mean
Time-period: July 1 to August 5, 1919										
	gm.	*gm.*	*gm.*	*hours*	*per cent*	*per cent*	*per cent*	°*C.*	°*C.*	°*C.*
July 8	82.6	153.6	70.9	100.6	79.5	38.1	58.8	33.2	14.0	23.6
July 15	113.5	130.9	17.5	49.4	80.7	37.5	59.1	29.2	11.3	20.3
July 22	76.0	98.2	22.2	69.5	80.8	41.1	60.9	32.0	15.1	23.5
July 29	109.1	130.7	21.6	68.4	79.8	43.4	61.6	32.7	14.1	23.4
August 5	83.4	112.7	29.3	58.1	80.1	39.2	59.6	27.0	11.5	19.3
Total	464.6	626.1	161.5	346.0						
Daily average	13.3	17.9	4.6	9.8	80.1	39.8	59.9	30.8	13.2	22.0
Time-period: November 23 to December 28, 1919										
November 30	96.5	106.9	10.4	8.5	75.5	56.7	66.1	22.0	14.6	18.3
December 7	114.5	137.7	23.3	17.7	76.5	45.1	60.8	25.3	11.6	18.5
December 14	112.5	123.2	10.7	14.6	76.2	50.2	63.2	23.6	13.1	18.4
December 21	109.1	125.0	15.8	31.3	75.2	44.0	59.6	24.8	11.8	18.3
December 28	109.6	120.6	11.0	16.7	70.2	37.4	53.8	28.0	12.7	20.4
Total	542.2	613.4	71.2	88.8						
Daily average	15.5	17.5	2.0	2.4	74.7	46.7	60.7	24.7	12.8	18.8
Time-period: January 21 to February 25 1920										
January 28	117.4	139.0	21.7	24.9	67.7	41.7	54.7	27.7	14.6	21.2
February 4	126.1	143.4	17.3	26.3	64.7	37.5	51.1	27.8	14.1	21.0
February 15	168.9	193.3	14.4	21.5	68.0	41.6	54.8	28.2	14.9	21.6
February 18	50.9	58.3	7.3	11.0	68.3	42.3	55.3	28.1	15.2	21.7
February 25	114.9	143.5	28.6	36.4	66.8	34.5	50.7	29.7	14.9	22.3
Total	574.1	677.5	99.4	120.1						
Daily average	16.5	19.4	2.8	3.3	67.1	39.5	53.3	28.3	14.7	21.5

* We are indebted to Mr. Morgan R. Sanford, of the U. S. Weather Bureau Station, for the figures from which the data of this column were computed.

Fresh weight of tops

All of the plant cut off just above the grain was considered as the top of the plant. All of the tops of a single culture were cut into approximately 2-cm. lengths and placed in a weighed test-tube. When all the tops of both series had been harvested in this manner, they were weighed immediately and the weight recorded as fresh weight of tops for each culture as a whole. Thus all of the values reported in this paper respecting any particular culture refer in every case to the entire six plants in that culture, and not to a single plant.

After the fresh weight of tops had been determined the tops were dried for 24 hours at a temperature of 78°C., after which they were dried to constant weight at 102°C. The test-tubes containing the tops were transferred from the oven to the desiccator and were allowed to cool before weighing. Each tube was stoppered with a rubber stopper of known weight while being weighed, the same rubber stopper being used for all the tubes.

The root systems of each single culture were placed together and excess liquid removed by blotting paper. They were then placed in a weighed test-tube and dried to constant weight in the same manner as described for the tops.

Dry weights of entire plants

These data are obtained as the direct summation of the actual dry weights of tops and roots. The calculated relative values are shown in table 3.

The first two columns of each time-period present the data for series A (the outer row on the rotating table) and series B (the inner row on the rotating table). The data as given in the tables are not the actual weights but the relative values of the weights expressed in terms of the weight of culture R_1S_1. The weight of culture R_1S_1 is considered as unity and its actual weight is given in parentheses, in grams. If it is desired to know the actual weight of any other culture of the series, it may be obtained by multiplying the relative weight of that culture by the actual weight of culture R_1S_1 as given in parentheses in the same column. The average seven highest cultures of each time-period are designated by H and the five cultures giving the lowest yields are designated by L.

Essentially the cultures showing the best yields of tops are the cultures which give the maximum weights of the entire plants.

To present graphically the data of table 3 use is made of triangle diagrams (fig. 2) similar to that shown in figure 1. The culture numbers are the same as those in figure 1 except that the numbers at the intersections within the triangle have been omitted as a matter of convenience. The areas with crosses on the triangle indicate the seven cultures having the highest dry weights. The areas with small circles indicate the five cultures having the lowest dry weights. The cultures having maximum and minimum weights are indicated

by large circles in their respective areas. It should be noted here that there is within the large triangle a smaller inner triangle composed of six solutions all of which offer favorable growth conditions. This triangle may be defined by giving the culture numbers at the apices, namely: R_2S_2, R_2S_4, R_4S_2. The areas of low yields are confined mainly to the lower apices of the large triangle.

TABLE 3

Dry weight of entire plants

Relative dry weights of series A and B and averages, grown at the three time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR_1S_1........	1.00L (2.10)	1.00L (1.96)	1.00L (2.03)	1.00L (0.676)	1.00L (0.658)	1.00L (0.667)	1.00L (1.53)	1.00L (1.59)	1.00L (1.56)
S_2........	1.14	1.09	1.11	1.24	1.19	1.22	1.19	1.15L	1.17L
S_3........	1.08	1.08L	1.08L	1.10	1.25	1.18	1.31	1.36H	1.34
S_4........	1.06	1.28H	1.17	1.06	1.12L	1.09L	1.04L	1.33	1.19
S_5........	1.11	1.34H	1.22	1.03	1.01L	1.02L	1.36H	1.46H	1.41H
S_6........	1.03	1.10	1.06L	0.97L	0.82L	0.90L	1.29	1.29	1.29
R_2S_1.........	0.98L	0.97L	0.94L	0.92L	1.32	1.12	1.18L	1.10L	1.14L
S_2.........	1.32H	1.16	1.24H	1.30H	1.32	1.31H	1.47H	1.35	1.41H
S_3.........	*1.55H*	1.39H	1.47H	1.25H	1.32H	1.29H	1.18L	1.45H	1.32
S_4.........	1.42H	1.03L	1.23	1.19	1.35H	1.27H	1.34	1.46H	1.40H
S_5.........	1.00L	0.92L	0.96L	1.00L	1.07L	1.04L	1.45H	1.27	1.36H
R_3S_1.........	0.98L	1.23	1.10	1.17	1.34H	1.26	1.27	1.09L	1.18
S_2.........	1.24H	1.33H	1.28H	1.40H	1.26	1.33H	1.46H	1.25	1.36H
S_3.........	1.36H	1.29H	1.33H	1.33H	1.19	1.26	1.53H	1.54H	1.54H
S_4.........	1.15	1.24	1.19	1.12	1.16	1.14	1.30	1.35	1.33
R_4S_1.........	1.31H	1.18	1.24H	1.21	1.28	1.25	1.28	1.06L	1.17L
S_2.........	1.40H	1.39H	1.40H	1.19	1.32H	1.26	1.52H	1.38H	1.45H
S_3.........	0.98L	1.29	1.13	1.02L	1.40H	1.21	1.32	1.15	1.24
R_5S_1.........	1.20	1.29H	1.25H	1.47H	1.30	1.39H	1.02L	1.25	1.14L
S_2.........	1.16	1.26	1.20	1.25H	1.34H	1.30H	1.67H	1.44H	1.56H
R_6S_1.........	1.14	1.11	1.13	1.47H	1.38H	1.43H	1.28	1.29	1.29
Shive's.......			1.08*	1.40	1.33	1.42*	1.32	1.26	1.21*
K...........				1.06	1.28	1.17	1.48	1.55	1.52
T............				1.11	1.26	1.19	1.82	1.59	1.71
Distilled H_2O.			0.16			0.05			0.23

* Average of three cultures.

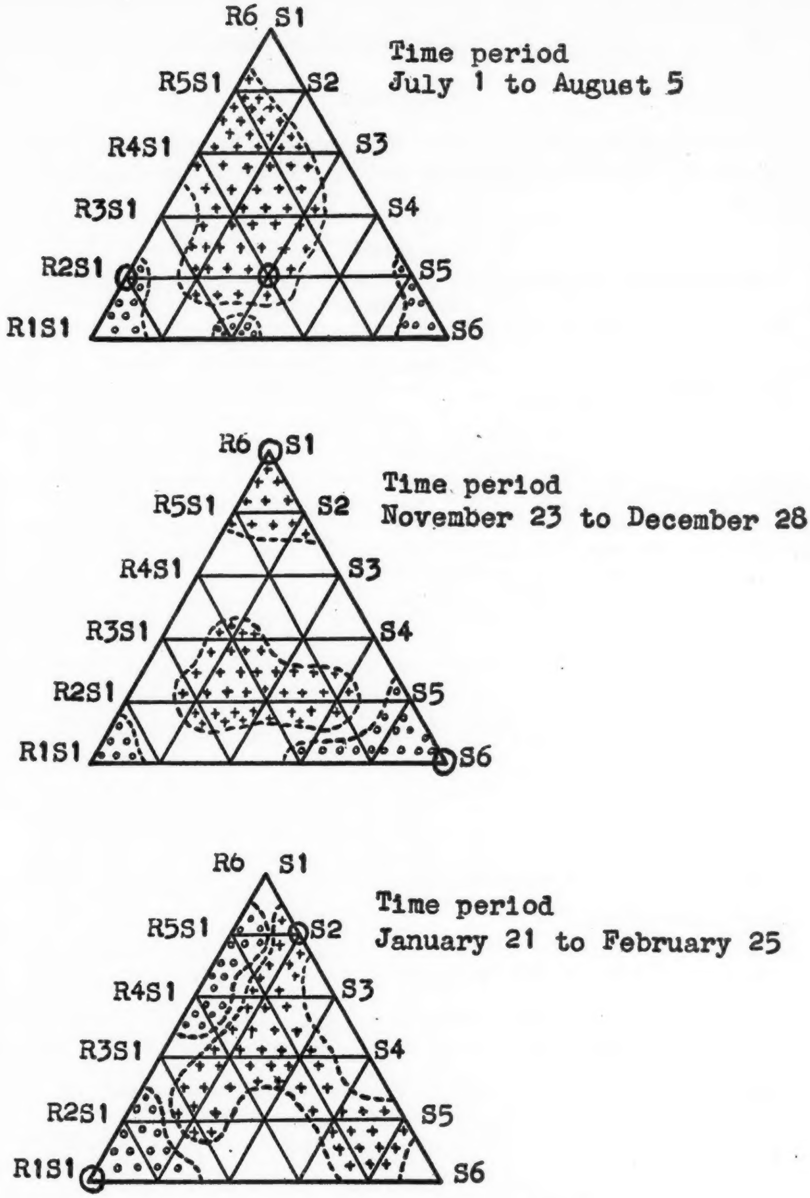

FIG. 2. DRY WEIGHT OF ENTIRE PLANTS

Diagrams showing the position of the cultures having the highest seven and lowest five relative yields, dry weight of entire wheat plants, averages of series A and B; high-yield areas are designated by crosses, areas of low yield by small circles; the cultures giving maximum and minimum yields are marked by large circles in their respective areas.

Transpiration

The total relative amounts of water (grams) absorbed by cultures are shown in table 4. These data are plotted on the triangle diagrams shown in figure 3.

TABLE 4

Transpiration

Relative amounts of transpiration of plants of series A and B, grown at three different time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR_1S_1........	1.00L	1.00L	1.00L	1.00	1.00	1.00	1.00L	1.00L	1.00L
	(878)	(908)	(893)	(422)	(411)	(416)	(728)	(680)	(704)
S_2........	1.09L	1.06	1.07L	1.09H	0.98L	1.04	1.10	1.10	1.10
S_3........	1.07L	1.03L	1.05L	0.97L	1.04	1.00	1.14H	1.18H	1.16H
S_4........	1.24H	1.16	1.20H	0.93L	0.97L	0.95L	1.08	1.11	1.10
S_5........	1.20	*1.25H*	1.22H	0.98	1.00	0.99	1.15H	*1.27H*	*1.21H*
S_6........	1.09	1.03	1.06L	0.89L	0.91	0.90L	1.16H	1.18H	1.17H
R_2S_1.........	1.01L	0.96L	0.99L	0.94L	1.04	0.99	1.03L	1.06	1.05
S_2.........	1.22	1.06	1.14	1.05H	1.09H	1.07H	1.11H	1.07	1.09
S_3.........	1.35H	1.15H	*1.25H*	1.10H	1.06H	1.08H	1.05	1.12	1.09
S_4.........	1.29H	1.01L	1.15	1.05H	*1.12H*	1.09H	1.01L	1.07	1.04L
S_5.........	1.14	1.05L	1.09	0.94L	0.92	0.93L	1.11	1.05L	1.08
R_3S_1.........	1.14	1.18H	1.16	0.99	1.12H	1.06H	1.10	0.94L	1.02L
S_2.........	1.17	1.12H	1.14	1.05	1.03	1.04	1.12H	1.13H	1.13H
S_3.........	1.19	1.07	1.13	1.14H	1.02	1.08H	1.09	1.15H	1.12H
S_4.........	1.17	1.13	1.15	0.96	0.92L	0.94L	1.03L	1.09	1.06
R_4S_1.........	1.23H	1.05	1.14	1.01	1.00	1.01	1.11	1.04L	1.08
S_2.........	1.28H	1.14	1.21H	0.98	0.97L	0.98L	1.12H	1.22H	1.17H
S_3.........	1.04L	1.15H	1.09	1.01	1.03	1.02	1.04	1.01L	1.03L
R_5S_1.........	1.21	1.15H	1.18H	*1.18H*	1.07H	1.13H	0.95L		0.95L
S_2.........	1.25H	1.12H	1.18H	0.99	1.05H	1.02	*1.17H*	1.07	1.12H
R_6S_1.........	1.24H	1.11	1.17H	1.14H	1.09H	1.11H	1.06	1.16H	1.11
Shive's.......			0.98*			0.97*	1.03	1.05	0.98*
K...........				0.93	0.95	0.94	1.05	1.10	1.08
T............				0.92	0.88	0.90	1.11	1.13	1.12
Distilled H_2O.						0.03			0.01

* Average of three cultures.

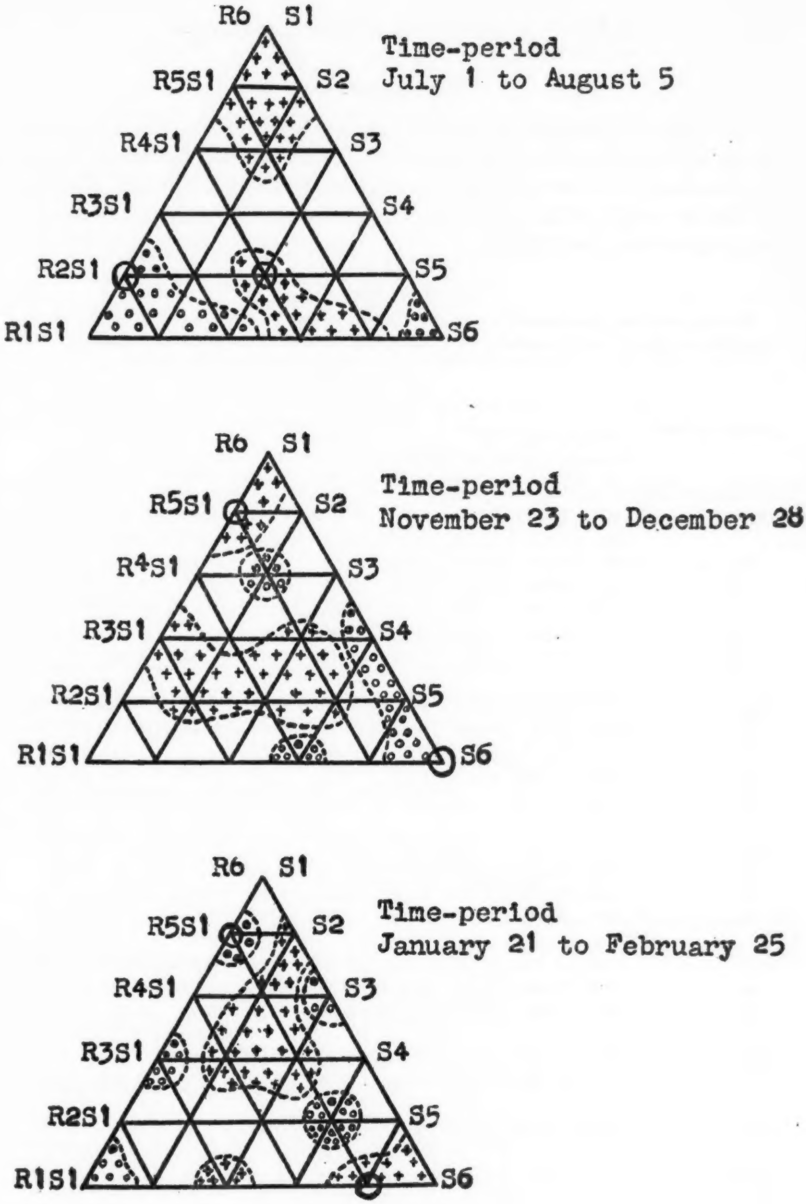

FIG. 3. TRANSPIRATION

Diagrams showing relative amounts of water lost by transpiration; areas of high transpiration indicated by crosses, areas of low transpiration indicated by small circles; the cultures showing the highest loss or lowest loss of water are designated by larger circles in their respective areas.

Water requirement

The water requirement of plants is commonly defined as the number of grams of water required to produce 1 gm. of dry weight of plant material. As calculated, it is the total number of grams of solution absorbed, divided by the dry weight of the entire plants. The data of the water-requirements are given in table 5 and figure 4.

TABLE 5

Water requirement

Showing relative amounts of water absorbed to produce 1 gm. of dry weight of plant material of series A and B and averages, conducted through three different time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR_1S_1	1.00	1.00H	1.00	1.00H	1.00H	1.00H	1.00H	1.00H	1.00H
	(414)	(463)	(438)	(625)	(626)	(625)	(476)	(427)	(451)
S_2	0.97	0.97	0.97	0.87	0.82	0.85	0.93H	0.96H	0.95H
S_3	0.99	0.96	0.98	0.89	0.83	0.86	0.87	0.86	0.87
S_4	1.18H	0.90	1.04H	0.88	0.86	0.87	1.03H	0.84	0.94
S_5	1.10	0.93	1.02H	0.96H	0.98H	0.97H	0.84	0.87	0.86
S_6	1.07	0.93	1.00	0.92	1.11H	1.02H	0.90H	0.92H	0.91H
R_2S_1	1.13H	0.99H	1.06H	0.99H	0.79L	0.89	0.87	0.96H	0.92H
S_2	0.94L	0.92	0.93L	0.81L	0.82	0.82L	0.75L	0.79L	0.77L
S_3	0.88L	0.83L	0.86L	0.88	0.80L	0.84	0.89	0.77L	0.83
S_4	0.90L	0.98H	0.94L	0.88	0.83	0.86	0.76L	0.73L	0.75L
S_5	1.15H	1.14H	1.15H	0.94H	0.87H	0.91H	0.76L	0.83L	0.80L
R_3S_1	1.17H	0.96	1.07H	0.85	0.82	0.84	0.86	0.92	0.89
S_2	0.95L	0.84L	0.90L	0.75L	0.81	0.78L	0.77L	0.91	0.84
S_3	0.88L	0.82L	0.85L	0.86	0.85	0.86	0.71L	0.75L	0.73L
S_4	1.03	0.91	0.97	0.86	0.80L	0.83L	0.79	0.81L	0.80L
R_4S_1	0.95L	0.89L	0.92L	0.84L	1.10H	0.97H	0.86H	0.96H	0.91H
S_2	0.92L	0.82L	0.87L	0.82L	0.73L	0.78L	0.74L	0.88	0.81L
S_3	1.07	0.90	0.99	0.99H	0.74L	0.87	0.79	0.88	0.84
R_5S_1	1.02	0.89L	0.96	0.81L	0.83	0.82L	0.93H	†	†
S_2	1.09	0.89L	0.99	0.79L	0.79L	0.79L	0.70L	0.74L	0.72L
R_6S_1	1.10H	0.99H	1.05H	0.78L	0.78L	0.78L	0.83	0.90	0.87
Shive's	0.97	0.91	0.94*	0.75	0.71	0.69*	0.78	0.83	0.82*
K				0.88	0.75	0.82	0.71	0.72	0.72
T				0.83	0.70	0.77	0.61	0.71	0.66
Distilled H_2O			0.63			0.67			0.74

* Average of three cultures.

† Container overturned.

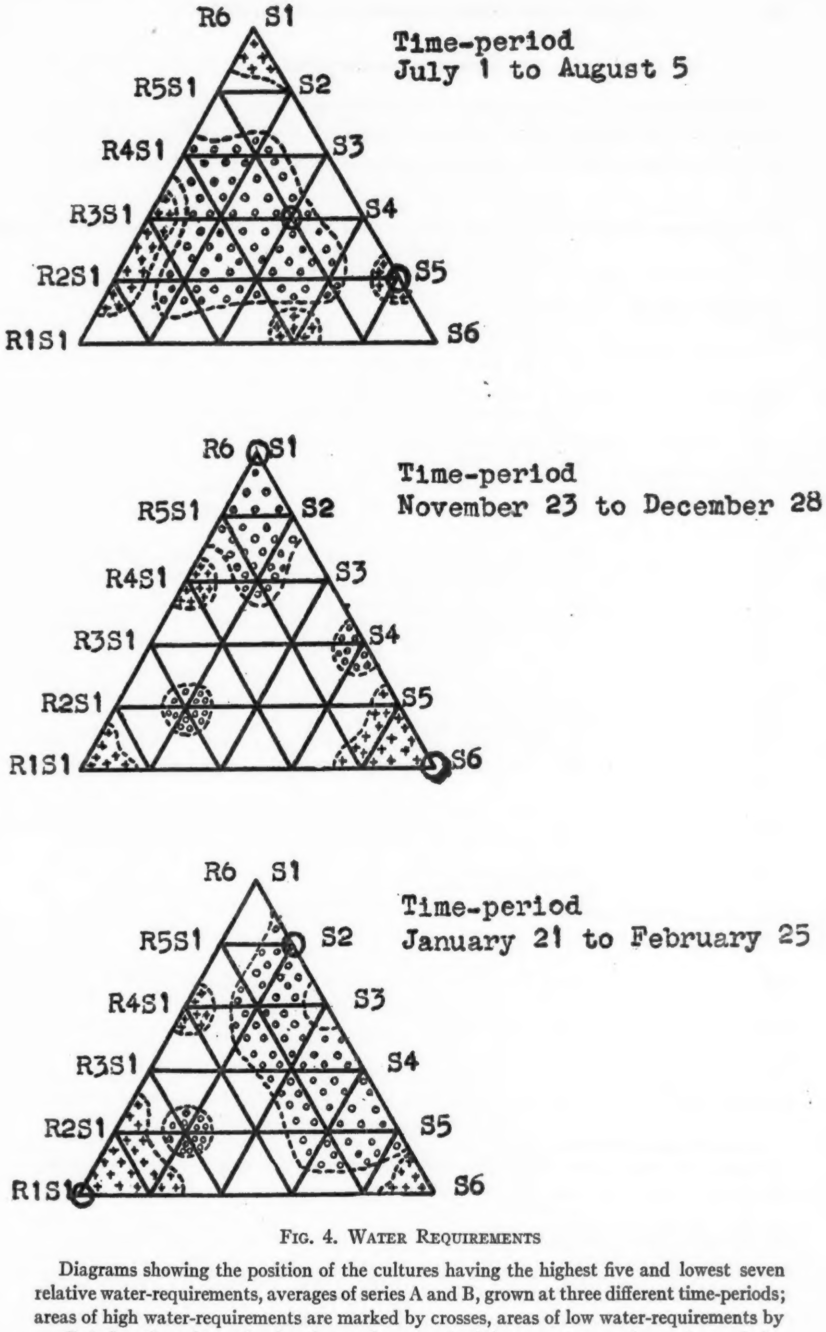

FIG. 4. WATER REQUIREMENTS

Diagrams showing the position of the cultures having the highest five and lowest seven relative water-requirements, averages of series A and B, grown at three different time-periods; areas of high water-requirements are marked by crosses, areas of low water-requirements by small circles; the cultures having the maximum and minimum water-requirements are designated by large circles in their respective areas.

Hydrogen-ion concentration

Measurements of the hydrogen-ion concentration were made of the nutrient solutions at the beginning of each time-period and again at the end of each 3½-day interval. The data thus obtained are presented in tables 6, 7 and 8.

TABLE 6

Showing changes of pH at the end of each 3½-day interval, averages of series A and B, conducted from July 1 to August 5, 1919, inclusive

SOLUTION NUMBER	JULY									AUGUST	
	1	4	8	11	15	18	22	25	29	1	5
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
R_1S_1	5.3	5.6	6.4	6.4	7.0	6.8	7.0	6.8	6.9	6.9	6.8
S_2	5.3	5.8	6.5	6.4	7.2	6.9	7.0	6.9	7.1	6.9	6.6
S_3	5.2	5.8	6.4	6.4	6.9	6.8	7.1	6.8	6.9	6.9	7.1
S_4	5.2	5.9	6.5	6.4	6.8	7.1	7.1	6.6	6.2	6.2	5.7
S_5	5.2	5.9	6.3	6.3	6.7	6.4	6.2	6.1	5.7	6.2	5.6
S_6	5.2	5.9	6.1	6.1	6.5	6.2	6.1	5.8	5.8	6.0	5.6
R_2S_1	5.0	5.4	6.1	6.1	6.5	6.5	6.3	6.3	6.2	6.3	6.1
S_2	4.9	5.5	6.2	6.2	6.4	6.3	6.2	6.1	6.1	6.6	5.8
S_3	4.9	5.5	6.1	6.1	6.2	6.1	5.8	5.7	5.5	6.2	5.1
S_4	4.9	5.4	6.1	6.0	6.0	5.9	5.6	5.6	5.4	5.8	4.9
S_5	4.9	5.6	5.9	5.9	5.8	5.8	5.4	5.5	5.3	5.5	5.2
R_3S_1	4.9	5.3	6.1	6.1	6.2	6.3	6.1	6.1	5.6	6.4	5.7
S_2	4.9	5.5	6.1	6.1	6.1	6.0	5.7	5.7	5.5	5.8	5.3
S_3	4.9	5.3	5.9	5.9	5.8	5.5	5.4	5.5	5.3	5.6	5.0
S_4	4.9	5.4	5.7	5.7	5.6	5.4	5.3	5.2	5.0	5.3	4.9
R_4S_1	4.9	5.3	5.9	6.0	6.1	6.1	5.8	5.9	5.9	6.2	5.7
S_2	4.9	5.2	5.9	5.9	5.9	5.8	5.4	5.4	5.3	5.5	5.0
S_3	4.9	5.3	5.7	5.5	5.5	5.3	5.2	5.1	5.0	5.3	4.9
R_5S_1	4.7	5.3	5.9	5.9	5.9	6.0	5.7	5.7	5.5	5.9	5.4
S_2	4.7	5.2	5.7	5.6	5.5	5.5	5.2	5.2	5.0	5.4	4.7
R_6S_1	4.9	5.2	5.9	5.9	5.8	5.9	5.6	5.5	5.4	5.6	5.2
Shive's*	4.6†	4.9	5.4	5.5	5.5	5.7	5.5	5.5	5.4	5.5	5.3
K											
T											
Distilled H_2O	5.8	6.4	7.0	6.3	6.3	6.7	6.3	6.3	7.8	7.1	6.2

* Averages of 3 cultures.

† Shive (15) reports the value, pH = 4.7; the difference between our values has little significance, since it is within the limit of experimental error for the colorimetric method

It will be observed that the difference between the initial and final pH (fig. 5) for a given 3½-day interval is greater when the nutrient solution contains only small amounts of the phosphate radical. Thus, the solutions along the basal

line of the triangle show the greatest changes in pH due, apparently, to the fact that they are insufficiently buffered by the lesser quantities of KH_2PO_4. While the solutions near or at the upper apex of the triangle are more highly

TABLE 7

Showing changes of pH at the end of each 3½-day interval, averages of series A and B, conducted from November 23 to December 28, 1919, inclusive

SOLUTION NUMBER	NOVEMBER			DECEMBER							
	23	26	30	3	7	10	14	17	21	24	28
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
IR_1S_1	5.3	5.5	5.6	6.1	6.5	6.0	6.3	6.1	5.8	5.4	5.4
S_2	5.3	5.4	5.6	6.1	6.7	6.1	6.4	6.1	6.1	6.0	5.5
S_3	5.2	5.4	5.5	6.1	6.6	6.2	6.5	6.2	6.1	5.4	5.2
S_4	5.2	5.5	5.6	6.1	6.7	6.1	6.5	6.1	6.0	5.8	5.4
S_5	5.2	5.5	5.6	6.2	6.6	6.2	6.4	6.1	6.0	5.6	5.6
S_6	5.2	5.5	5.7	6.3	6.3	6.1	6.1	6.0	6.0	5.9	5.7
R_2S_1	5.0	5.2	5.4	5.7	6.1	5.7	6.0	5.7	5.6	5.4	5.0
S_2	4.9	5.3	5.3	5.8	6.3	5.9	6.1	6.0	6.0	5.8	5.3
S_3	4.9	5.3	5.4	5.9	6.3	5.9	6.1	6.0	6.0	5.7	5.3
S_4	4.9	5.3	5.4	5.9	6.3	5.9	6.1	6.0	5.9	5.7	5.4
S_5	4.9	5.3	5.4	5.9	6.2	6.0	5.9	5.9	5.8	5.6	5.5
R_3S_1	4.9	5.1	5.3	5.8	6.1	5.6	5.9	5.8	5.7	5.5	5.1
S_2	4.9	5.1	5.3	5.5	6.1	5.6	6.0	5.9	5.8	5.7	5.1
S_3	4.9	5.2	5.3	5.7	6.1	5.8	6.0	5.9	5.7	5.5	5.0
S_4	4.9	5.2	5.3	5.6	6.1	5.7	5.7	5.7	5.6	5.5	5.4
R_4S_1	4.9	5.0	5.2	5.5	5.9	5.5	5.9	5.9	5.6	5.5	4.9
S_2	4.9	5.0	5.2	5.5	5.9	5.6	5.9	5.6	5.6	5.5	4.9
S_3	4.9	5.0	5.2	5.5	5.9	5.6	5.7	5.7	5.5	5.5	5.3
R_5S_1	4.7	4.9	5.1	5.5	5.9	5.5	5.8	5.7	5.6	5.5	4.8
S_2	4.7	4.9	5.1	5.5	6.0	5.6	5.9	5.6	5.5	5.4	4.9
R_6S_1	4.9	4.8	5.1	5.5	5.9	5.5	5.8	5.6	5.6	5.5	4.9
Shive's*	4.6†	4.6	4.7	5.2	5.5	5.2	5.5	5.4	5.3	5.4	4.7
K	5.1	5.1	5.3	5.9	6.1	5.8	5.9	6.3	6.2	5.5	5.4
T	4.9	4.6	5.1	5.4	5.8	5.4	5.7	6.1	5.9	5.1	4.8
Distilled H_2O	5.8	6.3	5.6	6.3	5.9	6.5	6.3	6.3	5.7	7.4	6.2

* Averages of 3 cultures.

† Shive (15) reports the value, pH = 4.7; the difference between our values has little significance, since it is within the limit of the experimental error for the colorimetric method.

buffered because of the increased quantity of the phosphate salt present, they are also more highly acid (the pH is less), because of the dissociation of the hydrogen-ion in this salt. These observations are in harmony with Salter

and McIlvaine. The reaction of all the solutions in which plants had been grown was toward the neutral point (pH = 7).[2]

TABLE 8

Showing changes of pH at the end of each 3½-day interval, averages of series A and B, conducted from January 21 to February 25, 1920, inclusive

SOLUTION NUMBER	JANUARY			FEBRUARY							
	21	25	28	1	4	8	11	15	18	22	25
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
IR_1S_1	5.3	5.6	6.2	6.6	6.7	6.5	6.7	7.0	6.7	6.9	6.4
S_2	5.3	5.7	6.2	6.6	6.8	6.6	6.7	7.1	6.9	7.1	6.8
S_3	5.2	5.4	5.9	6.2	6.4	6.2	6.3	7.3	6.9	7.5	7.2
S_4	5.2	5.6	6.1	6.4	6.7	6.6	6.7	7.1	6.7	7.4	6.8
S_5	5.2	5.8	6.3	6.6	6.5	6.2	6.3	6.6	6.3	7.2	6.8
S_6	5.2	5.7	6.3	6.5	6.3	6.1	6.4	6.6	6.4	7.4	6.5
R_2S_1	5.0	5.3	5.9	6.2	6.4	6.3	6.5	6.6	6.4	6.7	6.7
S_2	4.9	5.3	5.9	6.3	6.5	6.3	6.4	6.7	6.5	7.0	6.3
S_3	4.9	5.4	5.9	6.2	6.4	6.2	6.3	6.5	6.3	6.4	6.3
S_4	4.9	5.4	5.9	6.1	6.2	6.0	6.1	6.3	6.1	6.3	6.2
S_5	4.9	5.4	5.9	6.1	6.0	5.6	5.9	6.0	5.9	5.8	6.0
R_3S_1	4.9	5.1	5.7	6.0	6.1	6.0	6.3	6.5	6.4	6.2	6.2
S_2	4.9	5.3	5.8	6.0	6.2	6.1	6.3	6.4	6.2	6.1	6.2
S_3	4.9	5.3	5.7	6.0	6.1	5.8	6.0	6.1	5.9	5.8	5.7
S_4	4.9	5.2	5.6	5.9	5.9	5.4	5.6	5.6	5.4	5.3	5.3
R_4S_1	4.9	5.1	5.6	5.9	6.1	5.9	6.2	6.4	6.3	6.0	6.0
S_2	4.9	5.1	5.6	6.0	6.1	5.9	6.0	6.1	5.9	5.5	5.6
S_3	4.9	5.2	5.6	5.8	5.8	5.3	5.4	5.4	5.5	5.0	5.3
R_5S_1	4.7	5.0	5.4	5.8	6.0	5.8	6.1	6.2	6.1	5.8	5.7
S_2	4.7	5.1	5.6	5.8	5.8	5.5	5.6	5.5	5.5	5.3	5.5
R_6S_1	4.9	4.9	5.4	5.9	6.0	5.7	6.1	6.0	5.9	5.6	6.0
Shive's*	4.6†	4.8	5.1	5.4	5.6	5.4	5.6	5.6	5.5	5.5	5.5
K	5.1	5.4	5.9	6.1	6.0	5.8	6.1	6.3	6.1	6.0	5.9
T	4.9	5.0	5.4	5.8	5.7	5.5	5.5	5.6	5.4	5.1	5.3
Distilled H_2O	5.8	6.6	6.3	6.1	5.9	4.7	5.0	5.1	5.0	5.6	5.5

* Averages of 3 cultures.

† Shive (15) reports the value pH = 4.7; the difference between our values has little significance, since it is within the limit of the experimental error for the colorimetric method.

[2] This in general substantiates the observations of Itano (6), Hoagland (5), Salter and McIlvaine (12), and Duggar (2).

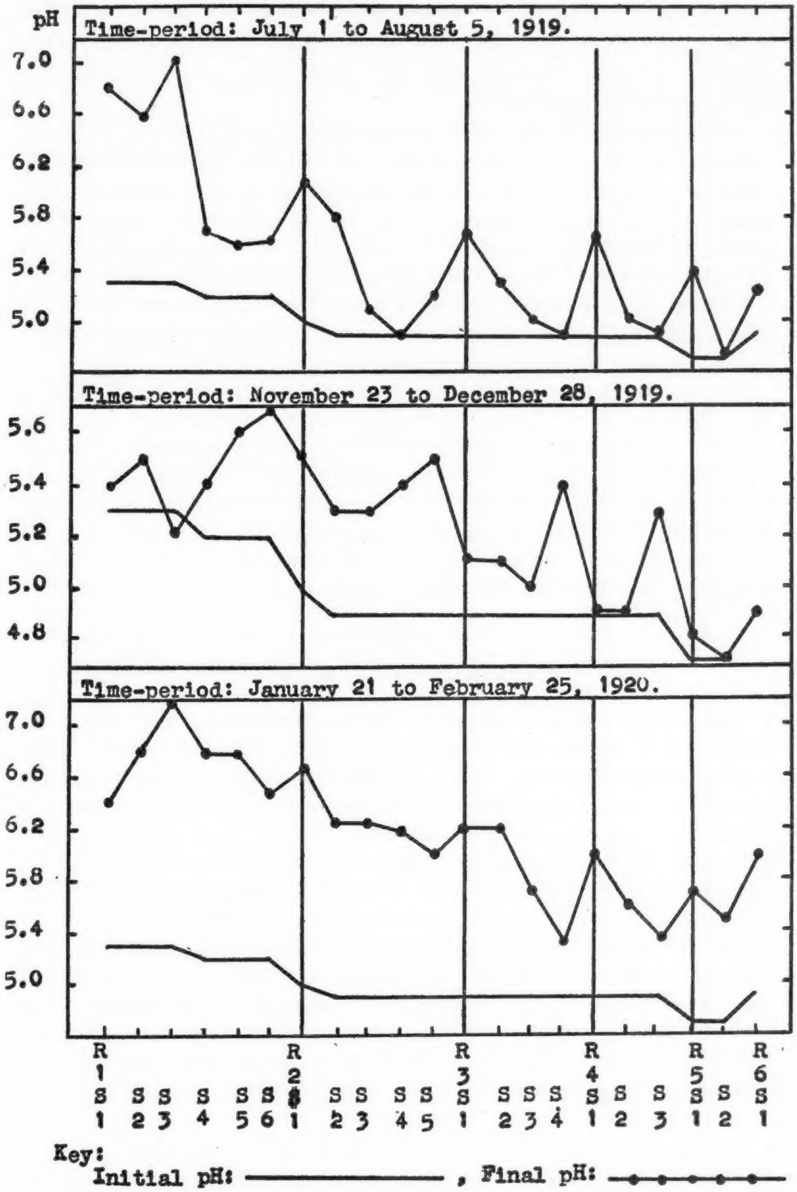

FIG. 5. DIAGRAMS SHOWING THE RELATION BETWEEN THE INITIAL pH VALUES, AND FINAL pH VALUES, AVERAGES OF SERIES A AND B, FOR THE THREE TIME-PERIODS

Condition of plants

The roots of the cultures throughout the three time-periods were fairly uniform. Roots of R_1S_1, R_1S_2, R_1S_3 were shorter, often of a brownish color, and had fewer secondary rootlets.

No very significant differences in size or condition of tops were noticeable until near the end of the growth period. At that time there were noticeable differences in height as well as apparent differences in vigor of plants; these differences were closely connected with the dry weights of cultures. The growth of the cultures during the second time-period was poor, due first of all to poor seasonal light conditions and second, to mildew infection.

Cultures of all three time-periods were infected to a greater or less extent with mildew but this infection never became a limiting factor except possibly in the cultures of the second time-period.

So-called magnesium or tip injury appeared in cultures R_1S_1, R_2S_1, R_3S_1, R_4S_1, R_5S_1, and R_6S_1. The greatest amount of this injury occurred in culture R_1S_1 and the injury declined progressively toward the upper apex.

It may be that the yellowing of the leaves of potato plants grown in sand-cultures as reported by Johnston (7) is due in part at least to an excess of magnesium, for his data show that the plants having the greatest approximate percentage of yellow leaf-area are the plants grown in solutions R_1S_1, R_2S_1, R_3S_1, etc. However, he makes no mention of the correlation.

DISCUSSION

Comparison of various weight data

The cultures of the first time-period having maximum and minimum yields, R_2S_3 and R_2S_1, respectively, show the same relative transpiring powers. During the second time-period there is agreement between the cultures showing minimum transpiration; though the culture having the highest transpiring power, R_5S_1, does not have the maximum weight. There is good general agreement between transpiration quantities and yield of plants during the third time-period. As an indicator of relative physiological activity, including growth, transpiration quantities are valuable; but their value rests chiefly in the corroborative evidence which they offer in support of other data. Our data as to the general relation between growth and transpiration support Shive's (14) conclusion that "water transpired appears to be as good a criterion as is the final dry weight, for judging the comparative growth obtained in the different solutions." The authors, however, have not found any direct correlation between high root-yields and low transpiration as he suggests.

Water-requirement and dry weight

It was noticed at the time the water-requirement data were calculated, that the cultures having the lowest water-requirements were the cultures that gave the total maximum yields of plants. A comparison of the triangles of the

three time-periods will show how consistently this relation held true. The areas showing high yields of plants correspond with the areas showing low water-requirements. The relation between transpiration and water-requirements is in general the same as for yield of plants and water-requirements; cultures having a high water-requirement have low transpiring powers and the minimum dry-weight yields.

Relation of ion ratios to yields

The high-yield areas (fig. 2) tend to concentrate themselves in the central portion of the triangle diagrams, whereas the maximum and minimum ion ratio values are grouped along the marginal lines of the triangles, leaving an unoccupied space in the center. Reference to table 9 giving the actual values

TABLE 9

Cation ratios—Values of the three cation ratios and the pH values of the nutrient solutions used in the experiments reported herein

SOLUTION NUMBER	Mg/Ca	Mg/K	Ca/K	pH*
IR_1S_1	*5.96H*	*5.96H*	1.00	5.3
S_2	2.51H	4.92H	1.96H	5.3
S_3	1.32	3.91H	2.95H	5.2
S_4	0.75	3.04H	4.04H	5.2
S_5	0.39L	1.95H	4.90H	5.2
S_6	0.16L	1.00H	*6.10H*	5.2
R_2S_1	4.88H	2.49H	0.51	5.0
S_2	2.02H	2.02H	1.00	4.9
S_3	1.00	1.51	1.51	4.9
S_4	0.50	1.00	2.00H	4.9
S_5	0.20L	0.51	2.53H	4.9
R_3S_1	4.04H	1.32	0.32L	4.9
S_2	1.50	1.00	0.66	4.9
S_3	0.66	0.66	1.00	4.9
S_4	0.24L	0.32L	1.32	4.9
R_4S_1	2.96H	0.74	0.25L	4.9
S_2	1.00	0.50	0.50	4.9
S_3	0.32L	0.24L	0.75	4.9
R_5S_1	2.04H	0.39L	0.19L	4.7
S_2	0.48	0.19L	0.39L	4.7
R_6S_1	1.00	0.16L	0.16L	4.9
Shive's	2.88	0.83	0.29	4.6

* Note: After this article had gone to press, McCall and Hagg reported (Soil Science, v, 10, p. 481–485) the pH values of all 6 types of solutions. It should be noted that their values for type I vary from ours by an approximate difference of 0.5 pH.

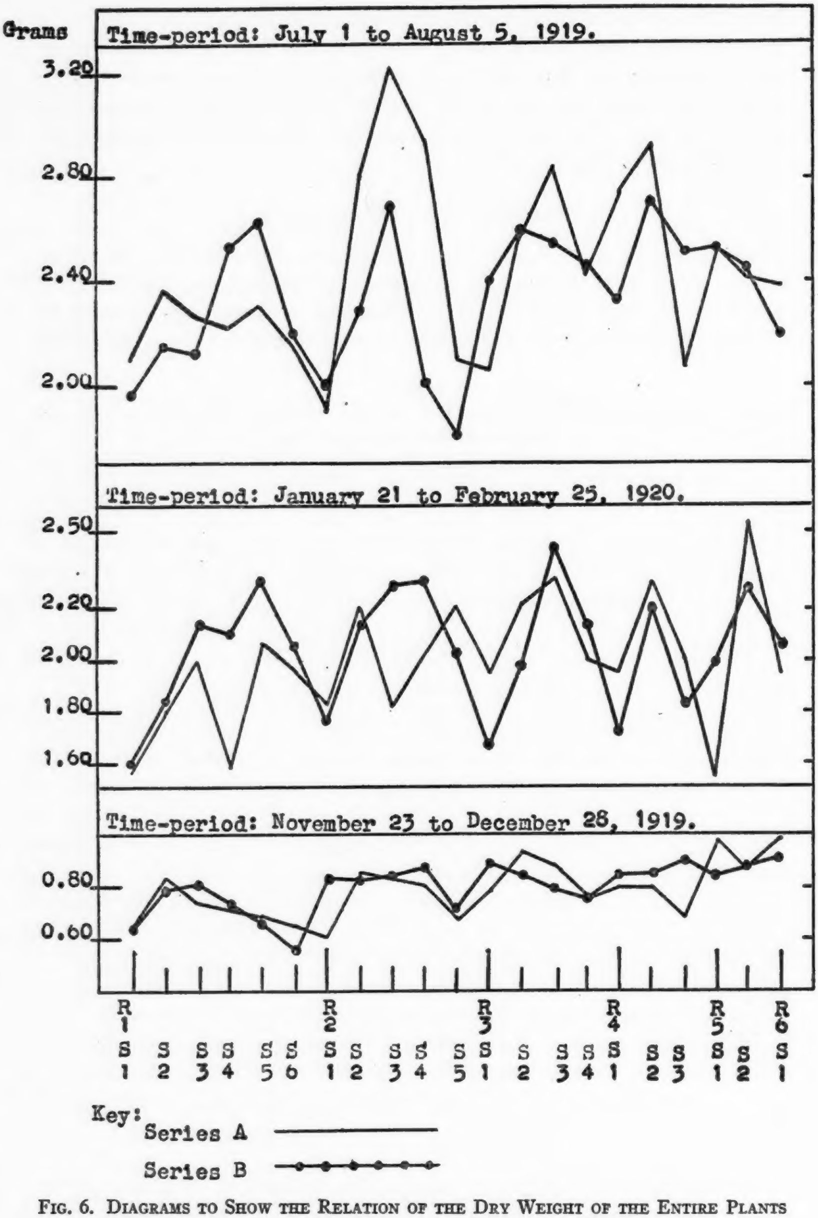

FIG. 6. DIAGRAMS TO SHOW THE RELATION OF THE DRY WEIGHT OF THE ENTIRE PLANTS OF SERIES A AND B, TO THEIR POSITION ON THE TRIANGLE DIAGRAM, AND THE CLOSE AGREEMENT BETWEEN THE TWO SERIES, A AND B, DURING EACH TIME-PERIOD

of these ratios will show that the solutions in this unoccupied central portion of the triangles have ratios of intermediate value with a tendency toward unity. Attention has been called to the fact that there seems to be an inner triangle, all the cultures of which, by reason of their salt balance, seem to offer optimum growth conditions. This inner triangle has as its apices cultures R_2S_2, R_4S_2, and R_2S_4. These solutions are all characterized by ion ratios which tend to become unity in respect to each other. The molecular proportions of the three salts of this inner triangle show this same relation thus:

		R_4S_2 2, 2, 4*		
	R^*S_2 2, 3, 3*		R^*S^* 2, 3, 3*	
R_2S_2 2, 2, 4*		R_2S_3 2, 3, 3*		R_2S_4 2, 2, 4*

Hydrogen-ion concentration and plant yields

A comparison of the hydrogen-ion concentrations of the solutions (fig. 5 and 6) giving high and low yields shows that the pH does not at least become a limiting factor, in spite of the rather high initial acidity of the solutions in the upper portion of the triangle. Solutions which are poorly buffered because of the small quantity of phosphate present and hence show maximum changes of pH during the 3½-day interval, are the solutions which support minimum-weight yields. It is doubtful in this instance that it is the absence of sufficient buffer material that results in poor-weight yields; rather it may be the absence of sufficient proper nutrient salts.

Hydrogen-ion concentration—transpiration

Figures 7, 8 and 9 show the amounts of solution absorbed during each 3½-day interval for each time-period. The number of grams of solution absorbed are plotted as ordinates, and the days on which the solutions were renewed, as abscissae. The pH of the solutions at the end of each 3½-day interval is plotted on the same diagrams, with the pH values as ordinates. Cultures R_1S_1, R_3S_3 and R_6S_1 are thus plotted.

The alteration in the reaction of the culture solutions in which the wheat plants had been grown increases with the age of the plants, for the first few weeks of growth. The alteration is due probably to either one of two things, the excessive withdrawal of certain ions from the solution, or the excretion of ions by the plants. The fact that for a time the increase in alkalinity of

* These figures refer to the molecular proportions of the three component salts (table 1). but for the sake of emphasis, the order of the arrangement of the components has been varied in some instances.

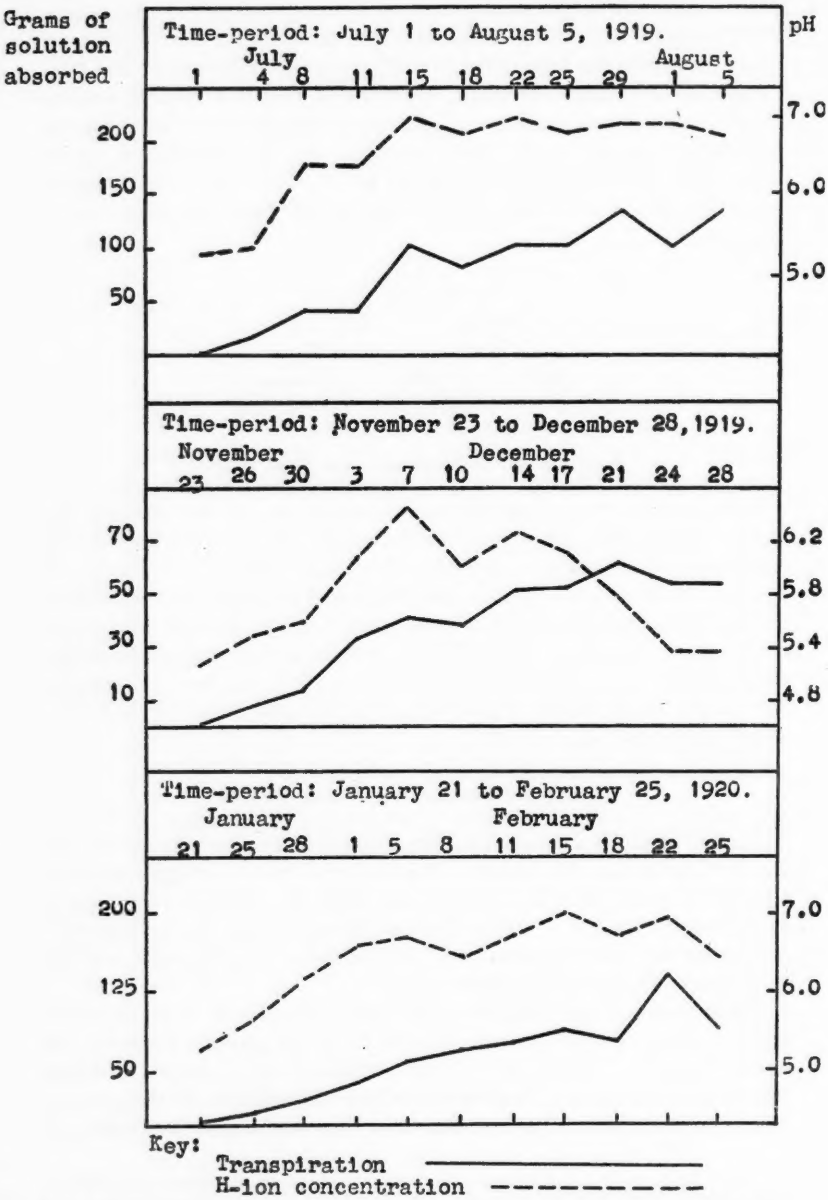

FIG. 7. DIAGRAMS SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE GRAMS OF SOLUTION ABSORBED BY R_1S_1, AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH 3½-DAY INTERVAL

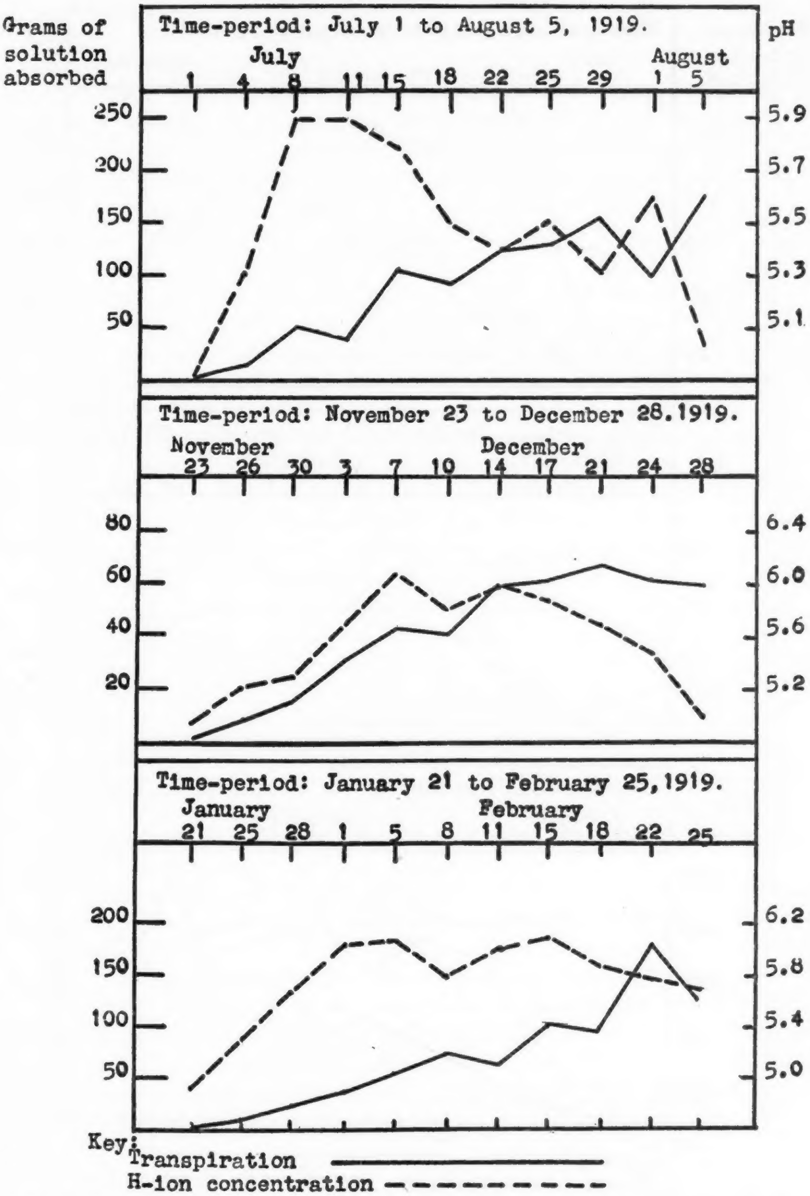

FIG. 8. DIAGRAM SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE NUMBER OF GRAMS OF SOLUTION ABSORBED BY R_3S_3, AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH 3½-DAY INTERVAL

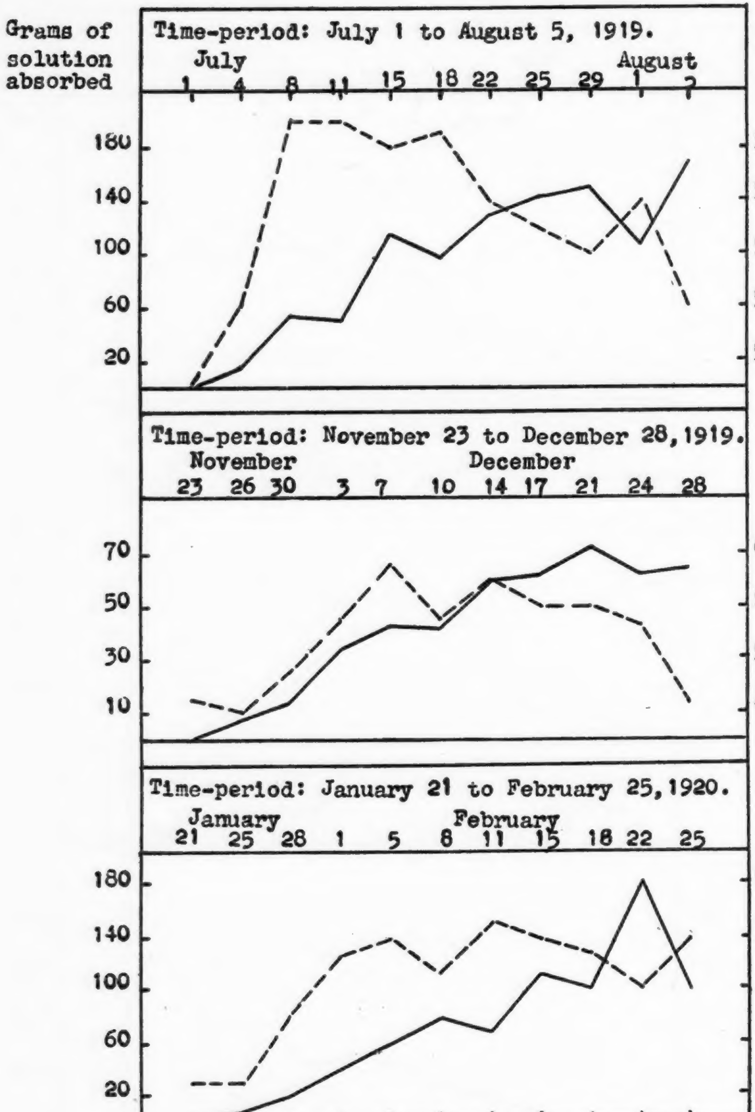

FIG. 9. DIAGRAM SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE NUMBER OF GRAMS OF SOLUTION ABSORBED BY R_6S_1, AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH 3½-DAY INTERVAL

the solutions tends to parallel the course of absorption would suggest that the change of pH may be due in part to the absorption of certain ions from the solutions.

The figures indicate that the ionic absorption, if such it is, apparently takes place in relatively the same manner in all of the solutions. A quantitative analysis of the solutions would in a large measure indicate whether or not the change in reaction was due in any way to the absorption of certain of the ions.

SUMMARY

1. Wheat plants were grown 5 weeks in duplicate series, during three different time-periods, in water-culture solutions composed of the three main salts varied in increments of $\frac{1}{8}$, all having an osmotic value of 1 atmosphere. The salts used were: KH_2PO_4, $MgSO_4$ and $Ca(NO_3)_2$, together with a "trace" of $FePO_4$.

2. Record was taken of the aerial conditions such as temperature, relative humidity, evaporating power of the air, effective radio-intensity, and daily hours of sunshine. The cultures grown during the first time-period, July 1 to August 5, were conducted under the most favorable environmental conditions, as stated in terms of total hours of sunshine and effective radio-intensity. The environmental conditions of the cultures grown during the third time-period, January 21 to February 25, were less favorable, when judged by the same criteria. The conditions under which the cultures of the second time-period, November 23 to December 28, were grown, were least favorable toward promoting maximum growth. All the plant-measurement data were shown to correspond to these seasonal variations.

3. No culture gave consistently maximum yields of tops, roots, or total dry weight throughout the three time-periods. Cultures R_2S_2, R_2S_3, R_2S_4, R_3S_2, R_3S_4, and R_4S_2 were usually included among the seven maximum cultures of each period. There was good agreement, however, between duplicate series of a given time-period.

4. Comparison of the water-requirements and the dry-weight yields show that the cultures having the maximum dry weights have the minimum water-requirements.

5. Data are presented which show that the hydrogen-ion concentration of solutions in which wheat has been grown tends to become less than the initial reaction of the solution. Thus all cultures were acid at the beginning and tended to become neutral. No data were obtained which explain the changes of pH in the solution. It was suggested that these differences may have been due to the selective absorption by the plant of certain ions from the solution.

6. There was no apparent direct correlation between the yield of the plants and the pH or the change of pH. Growth was generally less in those cultures (R_1S_1 and R_1S_6) which were poorly buffered, because of the insufficient quantity

of monopotassium phosphate present. They do show that degrees of acidity which have proven inhibitive to such microörganisms as *Actinomyces* and *Azotobacter* have no observable effect upon the growth of the wheat plant.

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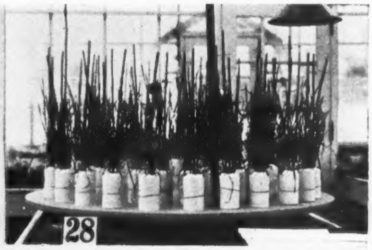
A



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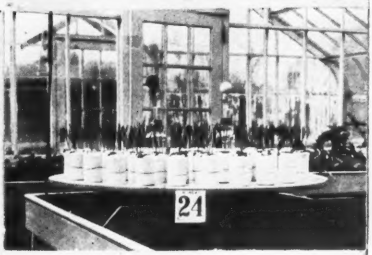

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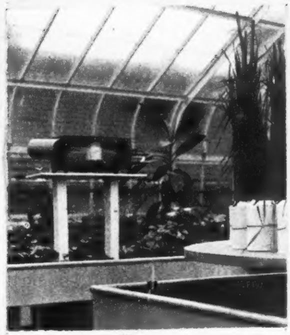

E

A. Cultures of series A and B on rotating table at the end of the first time-period, July 1 to August 5, 1919.

B. Cultures of series A and B on rotating table at the end of the second time-period, November 23 to December 28, 1919.

C. Cultures of series A and B on rotating table at the end of the third time-period, January 21 to February 25, 1920.

D. Cultures of series A and B on rotating table at the beginning of the second time-period, November 24, 1919. The black and white porous cup atmometers may be seen just above the tops of the cultures.

E. Showing the relative position of the thermohygrograph, on top of the two white supports, to the cultures on the rotating table, at the right.

ACID SOIL STUDIES: I. A STUDY OF THE BASIC EXCHANGE BETWEEN SOIL SEPARATES AND SALT SOLUTIONS

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INTRODUCTION

Most soils of Western Oregon, comprising chiefly the Willamette Valley and coast-lands are acid according to the usual methods now employed for the estimation of soil acidity. Field experiments show that while most of these acid soils respond to lime treatment, apparently some of them do not, as is indicated by the failure to get an increase in crop yields from the application of lime. Furthermore, some soils that may be judged neutral or only slightly acid respond well to lime treatment. A study of these different acid soils was undertaken with the hope that some differential factors might be disclosed that would explain the reason why some acid soils respond to lime treatment and others do not.

A phase of the work reported herein considers the reactions of several salt solutions on the soil separates that were segregated by a mechanical analysis of the samples. A review of the literature does not reveal any investigational work that has been done on the soil separates that would give light on the nature of the acidity, whether the sand fraction would show a greater or less acidity than the clay fraction or other points that might bear on the subject at hand.

PROCEDURE

Four characteristic acid soils were selected for the study and given the laboratory numbers 11076 to 11080, inclusive. The lime requirements determined by two methods and the classification according to the nomenclature of the Bureau of Soils, U. S. Department of Agriculture, are given in table 1.

Observations of field tests subsequently confirmed by pot experiments showed that applications of lime in different amounts to soil 11077 did not increase either legumes or grain crops.

The soil separates were obtained by a mechanical analysis of the different samples and each subdivision was isolated for study. In all samples there was only a very small amount of fine gravel which was subsequently discarded. The coarse and medium sand were combined and listed as coarse sand. The fine sand and the very fine sand were likewise combined and listed as fine sand. The silt or particles having a diameter between 0.005 to

0.05 mm. were separated from the clay particles in the usual manner and dried at room temperature. The clay or particles less than 0.005 mm. in diameter were obtained by transferring the soil solutions containing the clay in suspension to large shallow dishes and evaporating to dryness in the sunlight. Soil 11076 contained a very small amount of coarse sand which was added to the fine sand separate. The percentages obtained by this classification are given in table 2.

TABLE 1
Lime requirements of soils by two methods
Pounds of $CaCO_3$ per 2,000,000 pounds of soil

NUMBER	SOIL TYPE	VEITCH METHOD	JONES METHOD
		pounds	*pounds*
11076	Willamette silt loam	3,200	2,620
11077	Salem gravelly loam	1,500	2,160
11079	Clay loam	10,000	6,200
11080	Medium sandy loam	20,800	12,600

TABLE 2
The percentages of different separates in soils

NUMBER	COARSE SAND	FINE SAND	SILT	CLAY
	per cent	*per cent*	*per cent*	*per cent*
11076		28.0	52.1	19.9
11077	25.6	42.0	22.5	9.9
11079	28.5	26.5	29.2	15.8
11080	38.3	29.4	23.7	8.6

Five different salts, namely potassium nitrate, potassium chloride, potassium acetate, calcium acetate and sodium chloride, were used in the study. Approximately 0.1 *N* solutions of each of these salts were prepared. The exact amount of salt in 50 cc. of the 0.1 *N* solutions was determined by evaporating to dryness on the steam bath and finally to constant weight in the electric oven at 105°C. The amounts found were as follows:

KNO_3	0.4854
KCl	0.3745
$K(C_2H_3O_2)$	0.5024
$Ca(C_2H_3O_2)_2$	0.3900
$NaCl$	0.2900

Of the salt solutions prepared above 150-cc. portions were transferred to 250-cc. bottles and 15 gm. of the various soil separates were added to each respectively. The bottles were then agitated in a mechanical soil shaker for 3 hours and centrifuged at a high speed until all soil particles had settled and the supernatant liquid was clear. Aliquots of 50 cc. were then taken for

titration and for the determination of the salt content. The acidity liberated by the various salt solutions was determined by titration with 0.04 *N* sodium hydroxide with phenolphthalein indicator. Table 3 gives the number of cubic centimeters of 0.04 *N* sodium hydroxide that was required to neutralize the acidity of the soil separates liberated by the different salt solutions.

The 50-cc. portions taken for the determination of the salt content were evaporated to dryness in a flat-bottom dish and dried to constant weight in

TABLE 3

The amount of NaOH required to neutralize the acidity in 50 cc., employing the 0.1 N salt solution designated

SOIL NUMBER	SEPARATE	$K(C_2H_3O_2)$	KCl	KNO_3	$Ca(C_2H_3O_2)_2$	NaCl
		cc.	*cc.*	*cc.*	*cc.*	*cc.*
11076	Sand	2.50	0.03	0.03	3.00	0.03
11076	Silt	2.50	0.03	0.03	2.70	0.03
11076	Clay	4.50	0.20	0.24	5.30	0.10
11077	Coarse sand	2.00	0.06	0.06	2.44	0.03
11077	Fine sand	1.30	0.03	0.03	1.80	0.03
11077	Silt	2.44	0.14	0.06	3.10	0.03
11077	Clay	1.82	0.40	0.40	2.30	0.20
11079	Coarse sand	4.80	0.20	0.22	5.00	0.11
11079	Fine sand	4.50	0.20	0.20	4.60	0.11
11079	Silt	5.50	0.22	0.22	5.60	0.12
11079	Clay	5.20	0.38	0.40	5.00	0.22
11080	Coarse sand	11.42	1.80	1.40	12.00	0.42
11080	Fine sand	11.30	1.42	1.82	11.40	0.40
11080	Silt	9.80	1.36	1.50	9.40	0.42
11080	Clay	10.40	1.10	1.00	10.60	0.68

the electric oven at 105°C. Table 4 reports the actual amount of salt contained in 50 cc. of the salt solution used, the amount found after contact with the soil separates and the differences, that is, whether there was a greater or less amount of salt after contact with the soil separates than before. The plus (+) sign indicates that the quantity of salt in 50 cc. after contact with the separates was greater by the amount designated, than the amount contained in 50 cc. of the prepared solution; the minus (−) sign indicates the opposite to the plus sign, that is, a decrease in the amount found after contact with the soil particles.

TABLE 4

The amount of salts found in 50 cc. of solution before and after contact with soil separates

SOIL NUMBER	DESCRIPTION	POTASSIUM CHLORIDE			SODIUM CHLORIDE		
		Amount used	Amount found	Difference	Amount used	Amount found	Difference
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
11076	Sand	0.3745	0.4170	0.0425+	0.2933	0.3456	0.0523+
11076	Silt	0.3745	0.4118	0.0373+	0.2933	0.3320	0.0387+
11076	Clay	0.3745	0.4160	0.0415+	0.2933	0.3344	0.0411+
11077	Coarse sand	0.3745	0.4070	0.0325+	0.2933	0.3176	0.0243+
11077	Fine sand	0.3745	0.4036	0.0291+	0.2933	0.2972	0.0039+
11077	Silt	0.3745	0.3794	0.0049+	0.2933	0.2990	0.0057+
11077	Clay	0.3745	0.3988	0.0243+	0.2933	0.3300	0.0367+
11079	Coarse sand	0.3745	0.3868	0.0123+	0.2933	0.3398	0.0435+
11079	Fine sand	0.3745	0.3984	0.0239+	0.2933	0.3200	0.0267+
11079	Silt	0.3745	0.3998	0.0253+	0.2933	0.3250	0.0317+
11079	Clay	0.3745	0.4140	0.0395+	0.2933	0.3310	0.0377+
11080	Coarse sand	0.3745	0.3942	0.0207+	0.2933	0.3256	0.0323+
11080	Fine sand	0.3745	0.3878	0.0133+	0.2933	0.3134	0.0201+
11080	Silt	0.3745	0.4000	0.0245+	0.2933	0.3184	0.0251+
11080	Clay	0.3745	0.4270	0.0475+	0.2933	0.3482	0.0549+

SOIL NUMBER	DESCRIPTION	POTASSIUM ACETATE			CALCIUM ACETATE			POTASSIUM NITRATE		
		Amount used	Amount found	Difference	Amount used	Amount found	Difference	Amount used	Amount found	Difference
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
11076	Sand	0.4854	0.5066	0.0112+	0.3900	0.3926	0.0026+	0.5024	0.5100	0.0076+
11076	Silt	9.4854	0.4824	0.0016−	0.3900	0.3960	0.0060+	0.5024	0.5240	0.0216+
11076	Clay	0.4854	0.4806	0.0048−	0.3900	0.3832	0.0068−	0.5024	0.5150	0.0126+
11077	Coarse sand	0.4854	0.5046	0.0192+	0.3900	0.3814	0.0086−	0.5024	0.5160	0.0136+
11077	Fine sand	0.4854	0.5038	0.0184+	0.3900	0.3866	0.0034−	0.5024	0.5146	0.0122+
11077	Silt	0.4854	0.4828	0.0026−	0.3900	0.3856	0.0044−	0.5024	0.5040	0.0016+
11077	Clay	0.4854	0.4830	0.0024−	0.3900	0.3878	0.0022−	0.5024	0.5232	0.0232+
11079	Coarse sand	0.4854	0.4678	0.0176−	0.3900	0.3774	0.0126−	0.5024	0.5110	0.0086+
11079	Fine sand	0.4854	0.4814	0.0040−	0.3900	0.3802	0.0098−	0.5024	0.5116	0.0092+
11079	Silt	0.4854	0.4772	0.0082−	0.3900	0.3788	0.0112−	0.5024	0.5160	0.0136+
11079	Clay	0.4854	0.5106	0.0252+	0.3900	0.3836	0.0064−	0.5024	0.5230	0.0206+
11080	Coarse sand	0.4854	0.4646	0.0208−	0.3900	0.3628	0.0272−	0.5024	0.5038	0.0014+
11080	Fine sand	0.4854	0.4652	0.0202−	0.3900	0.3592	0.0318−	0.5024	0.4932	0.0092−
11080	Silt	0.4854	0.4762	0.0092−	0.3900	0.3636	0.0364−	0.5024	0.4944	0.0080−
11080	Clay	0.4854	0.4756	0.0098−	0.3900	0.3891	0.0009−	0.5024	0.5264	0.0240+

DISCUSSION

According to the results given in table 3, the acidity of the various separates of a certain soil was slightly different but the different salt solutions showed a wide range in the amount of acidity liberated. The greatest amount of acidity was set free by the salts of an organic acid, namely, potassium acetate and calcium acetate, while the salts of the inorganic acids, potassium nitrate, potassium chloride, and sodium chloride, gave much lower results. Similar observations have been made by other workers when salt solutions and the soil as a whole were used. It is interesting to note that the smaller particles or the clay separates which may be assumed to contain most of the soil colloids and organic matter, did not show a much higher acidity and in several cases less acidity than the coarse sand separates.

In table 4 we observe that the various salt solutions have a widely different effect on the soil separates as judged by the amount of the salt in solution after contact with the soil fractions. Here again we see a distinct difference between the action of salts of the organic and inorganic acids. Calcium-acetate and potassium-acetate solutions contained in almost every case a lower salt content after contact than before contact with the soil separates. The salts of the mineral acids, on the other hand, gave an increased amount after treatment with the soil particles. This was contrary to the results reported by Parker (3) in his work on selective adsorption by soils where he found a loss in salt content after contact with the soil. Furthermore, sodium chloride, although liberating the smallest amount of acidity, gave the highest salt content in solution after contact with the soil particles. In order to ascertain if possible the reason for the variation in the acidity of the soil separates, the causes for the difference in salt content of a solution after contact with the soil separates, and the manner in which the salts of organic and inorganic acids react, the experiment was repeated, and close observation of any significant reaction noted. Besides using 0.1 *N* salt solutions, normal solutions of the salts also were prepared and used in a similar manner to the weaker solutions. The salt content of the normal solutions was not determined after contact with the soil particles, since it was thought that the solvent effects and reaction of the stronger solutions on the soil particles would be so great that no reliable inferences could be drawn therefrom. Table 5 gives the number of cubic centimeters of 0.04 *N* NaOH required to neutralize the acidity liberated by normal salt solutions.

The presence of aluminum in a salt extract of an acid soil has long been known and reported by many workers. Likewise, during the titration of the potassium-chloride and potassium-nitrate solutions with sodium hydroxide after contact with the soil particles, it was observed that an appreciable amount of aluminum hydroxide and in most instances small amounts of iron hydroxide were precipitated out. The amounts varied somewhat for the separates of the different soils and may be summed up as follows: no. 11076

gave no visible amount of iron hydroxide; no. 11077, on the other hand, showed larger amounts of iron than aluminum, the quantity of the former varying somewhat in the different separates; the precipitate from no. 11079 and 11080 consisted chiefly of aluminum hydroxide with varying amounts of iron hydroxide in the separates. These observations were substantiated by the qualitative colorimetric method recently suggested by Comber (1) in which the characteristic red color of ferric thiocyanate was developed by treating the soil separates with alcoholic potassium-thiocyanate solution, the potassium of the potassium thiocyanate, displacing the iron which subsequently formed the color reaction. Furthermore, potassium-nitrate and chloride solutions after contact with soil 11080 showed only a slight acidity

TABLE 5

The amount of NaOH required to neutralize the acidity in 50 cc., employing the 1.0 N salt designated

SOIL NUMBER	SEPARATE	$K(C_2H_3O_2)$	KCl	KNO_3	$Ca(C_2H_3O_2)_2$
		cc.	*cc.*	*cc.*	*cc.*
11076	Sand	4.40	0.20		5.60
11076	Silt	4.00	0.10		5.00
11076	Clay	10.70	0.16		5.24
11077	Coarse sand	4.30	0.03		7.40
11077	Fine sand	2.40	0.03		4.30
11077	Silt	5.10	0.03		8.00
11079	Coarse sand	11.20	0.90	0.70	10.26
11079	Fine sand	9.90	0.90	0.70	10.40
11079	Silt	12.86	0.90	0.75	13.16
11079	Clay	12.30	0.94	0.72	
11080	Coarse sand	20.80	5.20	4.82	18.50
11080	Fine sand	19.40	4.12	4.80	19.10
11080	Silt	24.50	5.00	4.86	16.10

to methyl orange indicator, while after contact with the other soils the salt solutions were apparently neutral. Since salt solutions of aluminum chloride and aluminum nitrate are neutral to methyl orange and acid to phenolphthalein and appreciable amounts are present in the salt solution after contact with the separates, it is evident that the acidity liberated by salts of inorganic acids of the soil under observation is due to basic exchange in which the stronger basic elements, potassium and sodium, displace iron and aluminum and the latter pass into solution as nitrate or chloride depending upon the salt used for extraction, and may subsequently be titrated with sodium hydroxide, with phenolphthalein as an indicator. This conclusion was further substantiated by means of Truog's (6) zinc-sulfide method for acidity. It was observed in the Truog test for acidity in which calcium chloride and zinc sulfide is used and the degree of acidity present estimated by the intensity

of coloration of lead acetate paper, that the salts aluminum nitrate, aluminum chloride and ferric chloride gave similar indications of acidity depending upon the amount of salt used. Consequently, comparisons were made between the intensity of color produced by an amount of aluminum nitrate, approximately equal to the quantity of aluminum and iron hydroxide liberated from soil 11079 by a normal solution of potassium nitrate, and the intensity of color produced by the acidity of the soil as a whole. The intensity of the color produced by the aluminum nitrate and by the soil was approximately the same.

The chemical composition of the soil separates and the distribution of organic matter and soil colloids in the different separates may be factors that would influence the results reported in the tables above and thus account for any variation that appears abnormal. In a consideration of these points it may be assumed that most of the soil colloids are present in the finest particles or clay separates. The chemical composition of soil separates prepared from ten different samples has been investigated by Steinkoenig (5). The average amounts of silicon oxide, iron oxide, aluminum oxide and calcium oxide found in the various soil separates are given in table 6.

TABLE 6

The average amount of several constituents in soil separates

CONSTITUENT	SAND	SILT	CLAY
	per cent	*per cent*	*per cent*
SiO_2	88.50	83.05	45.52
Fe_2O_3	1.66	1.96	8.73
Al_2O_3	5.48	8.44	22.57
CaO	0.59	0.48	0.64

TABLE 7

Percentage of organic matter in the separates of a soil

SOIL NUMBER	COARSE SAND	FINE SAND	SILT	CLAY
	per cent	*per cent*	*per cent*	*per cent*
11079	4.60	3.67	5.95	6.06

The results indicate that the large particles or sand separates contain the highest amount of silicon oxide while the finer or silt separates contain higher amounts of calcium, iron and aluminum oxides. Failyer *et al* (2) report greater amounts of lime, magnesia, potash and phosphoric acid in the finer particles or clay separates, which was confirmed also by Steinkoenig (5). In regard to the distribution of organic matter it is probable that larger amounts will be found in the finer particles or clay separate. In order to confirm this assumption a determination of organic matter in the separates of soil 11079 was made. The Rather (4) hydrogen fluoride method was followed. The results are given in table 7.

The very fine particles or the clay and silt separates contain approximately the same amount of organic matter. The coarse sand, apparently, contains a higher amount of organic matter than the fine sand but this is probably due to the fact that small roots and other debris that were too large to be classified with the very fine sand were present in visible quantities in the coarse sand. When we consider, therefore, the differences in the composition of the various soil separates, the unequal distribution of colloids and organic matter, the molecular weight of the elements that take part in the basic exchange, and the solvent effect of the salt solutions, it is obvious that the slight variation in the acidity of the soil separates and the variation in the amount of salt found after contact with the separates, may be explained.

From the results obtained with the salts of the organic acid, namely, calcium acetate and potassium acetate, it is apparent that a different reaction has taken place from that indicated by the salts of mineral acids. A far greater amount of acidity was liberated while there was a decrease in the salt content of 50 cc. after contact with the soil separates. If the reaction had been similar, but more intense as indicated by higher acidity, to the reaction of the salts of mineral acids, a large increase in the salt content of the 50 cc. would be expected after contact with the soil separates. On account of the dissimilarity of the reactions another explanation was sought.

Examinations of the organic salt solutions after contact with the soil separates did not show the presence of iron and aluminum hydroxide when neutralized with sodium hydroxide, as was evident with the salts of inorganic acids. A determination of the calcium content of the calcium-acetate solution before and after contact showed that appreciable quantities of calcium had been taken up by the soil but no metallic elements had been displaced in the reactions. Furthermore, since there was a decrease in the salt content after reaction with the soil, it was thought that the calcium may have been selectively adsorbed or by basic exchange had replaced the hydrogen of hydrous silicates. In either case free acetic acid would be formed and by distillation could be quantitatively determined. Accordingly, 20 gm. of soil 11079 was introduced into a bottle containing 200 cc. of calcium-acetate solution, the calcium content of which had been accurately determined. The bottle was then shaken in a mechanical shaker for 3 hours, centrifuged to clarify the solution and filtered. Fifty-cubic-centimeter portions of the clear solution were then taken for the determination of the acidity liberated as indicated by titration immediately with sodium hydroxide, with phenolphthalein as an indicator, and 50 cc. for distillation which would give the acetic acid present. The distillation was allowed to proceed nearly to dryness and the distillate titrated with sodium hydroxide, with phenolphthalein as an indicator. Also 25-cc. aliquots were taken and the calcium content determined and calculated to calcium acetate. The results are given in table 8.

The results show that the calcium taken up by the soil from the calcium-acetate solution was equivalent approximately to the acetic acid liberated.

Furthermore, we see that the acidity of the distillate was the same as the acidity of the salt solution that had not been distilled. This indicates that all of the acidity liberated in the reaction was acetic acid. Considered from this standpoint, the reason for an actual decrease in the salt content of the organic salt solutions after reaction with the soil particles, can be understood. The calcium was removed from the salt solution and was replaced by hydrogen. The difference in the atomic weight of calcium and hydrogen would necessarily result in a smaller salt content even though the acetic acid were not removed during the evaporation process.

TABLE 8

Amount of calcium acetate in solution before and after contact with soil and the calcium acetate equivalent of the acetic acid liberated

SOIL NUMBER	BEFORE CONTACT	AFTER CONTACT	DIFFERENCE	ACID LIBERATED	ACIDITY OF DISTILLATE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
11079	0.3520	0.2982	0.0538	0.0484 (12.27 cc. 0.05 *N* NaOH)	0.0479 (12.12 cc. 0.05 *N* NaOH)

TABLE 9

H^+ ion concentration of soil separates

SOIL NUMBER	SEPARATE	$E \frac{N}{10}$	pH
11076	Sand	0.650	5.31
11076	Silt	0.650	5.31
11076	Clay	0.654	5.38
11077	Coarse sand	0.696	6.09
11077	Fine sand	0.686	5.92
11077	Silt	0.683	5.87
11077	Clay	0.683	5.87
11079	Coarse sand	0.658	5.44
11079	Fine sand	0.640	5.14
11079	Silt	0.668	5.61
11079	Clay	0.666	5.58
11080	Coarse sand	0.642	5.17
11080	Fine sand	0.644	5.21
11080	Silt	0.642	5.17
11080	Clay	0.640	5.14

The actual acidity of the soil separates as indicated by the hydrogen-ion concentration was determined by the gas-chain method. Three-gram portions of the different soil separates were transferred to small bottles that served as the electrode vessel. Two cubic centimeters of conductivity water was added to each separate and allowed to stand 24 hours. Immediately

before the determination was made, more water was added to obtain 3 gm. of soil to 30 cc. of water. The increase in potential due to the H^+ ion, was obtained by taking readings directly from a millivoltmeter. The voltage at 25°, $E \frac{N}{10}$, and the Sorensen pH values are given in table 9.

The results indicate that the hydrogen-ion concentration of different separates of the soils under observation is approximately the same. Apparently, therefore, the size of the soil particles, the presence of colloids or organic matter in the clay separates or the composition of the different separates does not have a great influence on the hydrogen-ion concentration.

SUMMARY

A study of the action of several salt solutions on the soil separates has been made.

The acidity of the different soil separates liberated by action of a certain salt solution is approximately the same.

The manner in which the salts of mineral acids, KNO_3, KCl and NaCl react with the soils studied, is apparently different from the salts of an organic acid, $K(C_2H_3O_2)$ and $Ca(C_2H_3O_2)_2$.

The so-called acidity liberated by potassium nitrate, potassium chloride, and sodium chloride was due mainly to aluminum and iron rendered soluble by basic exchange.

The acidity produced by calcium acetate and potassium acetate was due to acetic acid liberated either by replacement of the hydrogen of hydrous silicates or by selective adsorption of the basic element in the salt solution.

The hydrogen-ion concentration of different separates of the soil was constant.

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ACID SOIL STUDIES: II. CHANGES IN CALCIUM COMPOUNDS ADDED TO ACID SOILS

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This paper deals with another phase of the problem reported in the foregoing article (2) in which studies were made to ascertain, if possible, the reasons why some acid soils of Oregon do not respond to lime treatment. The question now arises regarding the changes that calcium carbonate undergoes, when applied to an acid soil, if it does not function in correcting the acid condition; or if it does neutralize the acidity, in what manner is the reaction or chemical combination different from that exhibited by calcium carbonate applied to an acid soil that does respond to lime treatment? In an effort to answer this question a study was made of the changes that occurred when certain calcium compounds were added to the two types of acid soils.

The same so-called acid soils were used in this work as in the study reported in the foregoing paper (2). The same laboratory numbers, namely 11076, 11077, 11079 and 11080 were maintained. Further information regarding classification, lime requirement, or other points in question may be ascertained by reference to paper I (2).

A sufficient amount of each of the different soils was prepared to fill pots having a capacity of approximately 3 gallons. Chemically pure calcium carbonate and calcium oxide, prepared by igniting pure calcium carbonate, were used in amounts equivalent to the lime requirement of the different soils. Soil 11077 which, according to field observations, did not respond to lime treatment, received additional treatments in which calcium sulfate was used in amounts equivalent to the lime requirement and calcium carbonate was used in an amount that was double the lime requirement. After thoroughly incorporating the calcium compounds with the dry soil it was transferred to the pots. Control pots containing each of the different soils untreated also were prepared. Table 1 gives the amounts of different soils used and the treatments.

The pots containing the soil treated in the manner described above were then sunk into the ground level with the top of the pots and exposed to the weather. A crop of spring barley was grown in all pots to aid natural functioning and changes that the calcium compounds might undergo.

After exposure to the weather for one year, representative samples, taken to the depth of 6 inches, were removed from each pot. The forms into which

the calcium compounds had changed were then determined by the methods suggested by Shorey, Fry and Hazen (3). The methods, changed to suit the work at hand, are as follows:

The total calcium was determined by decomposing 5 gm. of soil with 10 gm. of sodium peroxide at low heat. The mass was then taken up with water, acidified, and the calcium percipitated as oxalate in an aliquot after the removal of iron and aluminum.

The carbon dioxide was liberated and absorbed in barium hydroxide according to the method outlined by Truog (4).

Water-soluble calcium oxide and sulfur trioxide were determined in the water extract obtained by shaking 40 gm. of soil with 200 cc. of water and centrifuging at a high speed until a clear solution was obtained.

TABLE 1

Treatment of soils

SOIL NUMBER	WEIGHT OF SOIL USED	COMPOUND ADDED	AMOUNT ADDED
	gm.		*gm.*
11076	12000	Control	
11076	13000	$CaCO_3$	21.8
11076	12800	CaO	12.9
11077	12500	Control	
11077	12500	$CaCO_3$	11.4
11077	12000	$CaCO_3$	22.3
11077	12000	CaO	6.2
11077	12000	$CaSO_4.2H_2O$	19.1
11079	12700	Control	
11079	12700	$CaCO_3$	65.3
11079	12700	CaO	37.2
11080	11000	Control	
11080	11000	$CaCO_3$	59.6
11080	11000	CaO	33.5

The acid-soluble calcium was determined by two methods designated "A" and "B," respectively, by Shorey, Fry, and Hazen (3). In method "A" the soil was digested with 4 per cent hydrochloric acid; in method "B" it was leached with 2 per cent hydrochloric acid. After washing the soil free of acid the calcium was determined in the respective filtrates.

The data obtained by these methods are given in table 2.

The interpretation of the analytical data reported above in terms of calcium compounds formed assume certain combinations. The schematic presentation and system of calculation as employed by Shorey, Fry and Hazen (3) was followed. Table 3 gives the percentages of various hypothetical calcium compounds that were present in the treated and in the control soils.

TABLE 2

The percentages of constituents obtained by the method employed

SOIL NUMBER	TREATMENT	TOTAL CaO	ACID-SOLUBLE CaO		WATER-SOLUBLE CaO	WATER-SOLUBLE SO_3	CARBON DIOXIDE
			4 per cent HCl	2 per cent HCl			
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
11076	Control	1.05	0.37	0.38	0.012	0.008	Negative
11076	$CaCO_3$	1.12	0.51	0.47	0.013	0.006	Negative
11076	CaO	1.12	0.45	0.45	0.020	0.009	Negative
11077	Control	2.60	0.20	0.10	0.015	0.004	Negative
11077	$CaCO_3$ (11.4 gm.)	2.68	0.46	0.13	0.018	0.005	Negative
11077	$CaCO_3$ (22.3 gm.)	2.75	0.69	0.16	0.020	0.004	Negative
11077	CaO	2.76	0.34	0.10	0.020	0.005	Negative
11077	$CaSO_4$	2.75	0.49	0.10	0.017	0.005	Negative
11079	Control	0.27	0.18	0.17	0.018	0.009	Negative
11079	$CaCO_3$	0.60	0.45	0.45	0.018	0.007	Negative
11079	CaO	0.56	0.34	0.33	0.013	0.008	Negative
11080	Control	2.06	0.45	0.43	0.022	0.014	Negative
11080	$CaCO_3$	2.45	0.89	0.60	0.030	0.012	Negative
11080	CaO	2.45	0.79	0.59	0.039	0.007	Negative

TABLE 3

Hypothetical calcium combinations in treated and untreated soils

SOIL NUMBER	TREATMENT	TOTAL CaO	CaO AS $CaCO_3$	CaO AS EASILY DECOMPOSABLE SILICATE	CaO AS DIFFICULTLY DECOMPOSABLE SILICATE	CaO WITH HUMUS	CaO AS $CaSO_4$
		per cent		*per cent*	*per cent*	*per cent*	*per cent*
11076	Control	1.05	None	0.00	0.68	0.370	0.010
11076	$CaCO_3$	1.12	None	0.04	0.62	0.460	0.010
11076	CaO	1.12	None	0.00	0.67	0.440	0.010
11077	Control	2.60	None	0.11	2.40	0.084	0.006
11077	$CaCO_3$ (11.4 gm.)	2.68	None	0.33	2.22	0.123	0.007
11077	$CaCO_3$ (22.3 gm.)	2.75	None	0.53	2.06	0.154	0.004
11077	CaO	2.76	None	0.24	2.42	0.095	0.007
11077	$CaSO_4$	2.75	None	0.39	2.26	0.095	0.007
11079	Control	0.27	None	0.01	0.09	0.160	0.010
11079	$CaCO_3$	0.60	None	0.00	0.15	0.440	0.010
11079	CaO	0.56	None	0.01	0.22	0.320	0.010
11080	Control	2.06	None	0.02	1.61	0.410	0.020
11080	$CaCO_3$	2.45	None	0.29	1.56	0.580	0.020
11080	CaO	2.45	None	0.20	1.46	0.580	0.010

A comparison of the total calcium-oxide content of the treated soils with the total calcium oxide of the controls shows that most of the calcium had been retained, although no doubt part had been lost by leaching since about 40 inches of rainfall had occurred during the year. Furthermore, there was a complete change to other combinations from the form in which the calcium had been added. In no case was any calcium carbonate present as indicated by the negative result for carbon dioxide. In the soil that had been treated with calcium sulfate no increase in water-soluble sulfur trioxide was obtained, which indicates that all sulfates had been lost by leaching Since, however, some of the calcium had been retained by the soil, it is probable that as the calcium sulfate dissolved, the calcium that was retained was taken up by the soil by basic exchange, while the sulfate was leached out in the substituted form.

TABLE 4

Acidity, by the Jones method, of treated and untreated soils after exposure to weather for one year

SOIL NUMBER	TREATMENT	0.04 *N* NaOH
		cc.
11076	Control	3.20
11076	$CaCO_3$	1.70
11076	CaO	2.10
11077	Control	3.00
11077	$CaCO_3$ (11.4 gm.)	2.40
11077	$CaCO_3$ (22.3 gm.)	1.53
11077	CaO	2.45
11077	$CaSO_4$	3.00
11079	Control	6.80
11079	$CaCO_3$	2.70
11079	CaO	3.60
11080	Control	12.90
11080	$CaCO_3$	4.10
11080	CaO	4.30

With the exception of soil 11077, most of the calcium added to the different soils was combined with humus. Since in most cases there was a higher water-soluble calcium content in the treated soils than in the controls, it is apparent that the calcium with humus would probably be more easily available and would provide an optimum medium for the development of favorable soil organisms. On the other hand soil 11077 which does not respond to lime treatment, showed that very little of the added calcium combined with humus, but was used to form easily decomposable silicates that are soluble in 4 per cent hydrochloric acid but not in 2 per cent hydrochloric acid. Where calcium was added to this soil in an amount that was double the lime requirement, more of the calcium combined with humus.

There was no marked difference between the reactions of calcium carbonate and calcium oxide or in the compounds formed.

Observations were made on the change in the lime requirement as indicated by the Jones (1) method for soil acidity. Table 4 reports the acidity liberated, in terms of cubic centimeters of 0.04 N sodium hydroxide.

The results indicate a reaction in which acid is liberated, although an excess of calcium carbonate and calcium oxide had been added to the treated soils. It is noticeable in soil 11077 where 22.3 gm of calcium carbonate, or double the lime requirement, had been added that the acid liberated was lower than where 11.4 gm had been added.

Since the Veitch (5) method was employed as a criterion of the lime requirement, tests were made to ascertain whether the soils after treatment and exposure to the weather for one year would react acid, as indicated by the Jones method. It was found that for every soil treated with calcium carbonate or calcium oxide an alkaline reaction was obtained It is evident, therefore, that since water extracts of the treated soils were alkaline as determined by the Veitch test, the so-called acidity of these soils may be considered neutralized after exposure to the weather for one year.

CONCLUSIONS

When calcium carbonate or calcium oxide was added to several acid soils the calcium retained after exposure to the weather for one year was combined chiefly with humus (organic matter) and easily decomposable silicate.

Most of the calcium present in the acid soil that does not respond to lime treatment was found combined as difficultly decomposable silicate. The calcium added was combined chiefly as easily decomposable silicate. This, however, does not explain the reason why the soil does not respond to lime treatment.

After exposure to the weather for one year all of the soils treated with either calcium carbonate or calcium oxide were alkaline according to the Veitch test.

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THE INFLUENCE OF FERTILIZERS CONTAINING BORAX ON THE YIELD OF POTATOES AND CORN—SEASON 1920[1]

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Several reports (3, 5)[2] have appeared concerning injury to the potato crop, during the season of 1919, by borax which occurred as an impurity in certain commercial potash salts obtained chiefly from Searles Lake, Cal. Reports (1, 2, 4) also have been issued concerning injury to other crops the same season, along with reports of investigational studies on the subject.

As the result of certain control measures, as well as careful supervision on the part of the companies producing the potash salts, no authentic case, so far as known, of injury to crops by borax was reported in 1920.

Certain points having arisen, however, it seemed desirable to conduct field investigations in order to ascertain: (a) the effects of different concentrations of borax upon the growth and yield of a number of crop-plants; (b) the influence of rainfall and soil type upon the effect of borax; and (c) the influence of time and method of application of a fertilizer mixture containing added borax in varying concentrations, upon the growth and yield of the selected crop-plants.

Following out this idea the Bureau of Plant Industry of the U. S. Department of Agriculture, through Dr. Oswald Schreiner, in charge of Soil Fertility Investigations,[3] asked the New Jersey Agricultural Experiment Station to coöperate in some experiments in which varying amounts of borax should be introduced in a control fertilizer, to be used on potatoes and corn.

The control fertilizer was a mixture of cottonseed meal, acid phosphate and muriate of potash analyzing 4 per cent ammonia, 8 per cent phosphoric acid and 4 per cent potash. The borax was mixed with this fertilizer in such proportions as to make the anhydrous borax application, in pounds per acre, as

[1] Paper No. 20 of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology, cooperating with the United States Department of Agriculture.

[2] Also articles appearing in the agricultural press.

[3] Other cooperative stations were established in Maine, Virginia, and Alabama under the direction of the Office of Soil Fertility Investigations.

follows: 1, 2, 3, 4, 5, 10, 20, 50, 100, 200 and 400. To simplify the work, the plot number was made to correspond with the number of pounds of borax per acre; thus plot 1 corresponds to 1 pound of borax with 1500 pounds of the standard fertilizer per acre; plot 2 to 2 pounds of borax and 1500 pounds of fertilizer per acre, etc. Each plot consisted of two rows of potatoes (one row of corn) the full length of the strip.

Check rows, that is, those which receive fertilizer without borax, were introduced as follows: check 1 outside of plot 1; check 2, between plots 3 and 4; check 3, between plots 10 and 20, and check 4 between plots 50 and 100. This arrangement provided a fair distribution of checks over the area and thus made it possible to interpret results even though there is some lack of uniformity in the soil.

The soil type on which this experiment for both corn and potatoes was conducted is a Sassafras loam of good quality.

In order that a test might be made of the influence of time and method of applying the fertilizers, the plot of ground was divided crosswise into three equal sections with the provision that the fertilizer should be drilled in the furrow on section 1 some two or three weeks before planting the crop; on section 2 it was to be drilled in the furrow at the time of planting, and on section 3 spread broadcast over the furrow at the time of planting.

THE POTATO CROP

For the potatoes the fertilizer was applied in the furrow by hand to section 1 on April 16; it was slightly mixed with the soil by passing a hoe along the bottom of the furrow. It was left in this condition until April 27 when the applications were made to sections 2 and 3 as above noted and potatoes planted on all sections. On sections 2 and 3 the fertilizer was slightly mixed with the soil, as in the case of section 1, before the potatoes were dropped. Covering was done by means of a horse cultivator.

This method of applying fertilizer and planting, it was thought, would clear up the question as to whether early application of the fertilizer might partially or wholly overcome the injurious action of the borax, which had been noted when fertilizer was applied at the time of planting the potatoes.

With the exceptions noted, that is, the time and method of applying the fertilizers, the three sections were treated exactly alike. The potatoes received the usual attention in the way of cultivation, hoeing and spraying. They were kept free from grass and weeds until toward the end of the season when, on account of much rain, grass made considerable headway on all plots except those which received the heavy applications of borax.

The weights of the potatoes, by plots, for the three sections, are shown in table 1. The table is best studied section by section and since the "seconds" form a rather small percentage of the total weight, the conclusions will probably be the same whether one considers the weight of the "primes" or the total weights.

Section I

When the weights for the different plots are considered with reference to the check plot nearest any given plot, it would appear that there is little or no decrease in yield for this section with as much as 50 pounds of borax per acre. It may be pointed out that there is actually a decline in yield from check plot 3 on through the plots that received 20, 30 and 50 pounds of borax per acre, but check 4 which lies along side of the plot receiving 50 pounds of borax yielded a total of only 72.8 pounds of potatoes as against 71.5 pounds for the plot with 50 pounds of borax. From this, it would appear that the gradual decline in yield from check 3 to check 4 was due to some other factor

TABLE 1

Yield of potatoes in coöperative borax experiment

QUANTITY OF BORAX PER ACRE	SECTION 1			SECTION 2			SECTION 3		
	Primes	Seconds	Total	Primes	Seconds	Total	Primes	Seconds	Total
lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
0 (Check 1)	78.25	7.50	85.75	75.50	5.50	81.00	58.25	7.50	65.75
1	64.20	5.75	69.95	56.30	6.85	63.15	41.40	5.00	46.40
2	75.55	4.65	80.20	72.50	9.00	81.50	58.05	5.80	63.85
3	75.65	6.65	82.30	65.60	9.10	74.70	60.15	5.75	65.90
0 (Check 2)	79.75	7.70	87.45	62.95	10.35	73.30	65.70	4.45	70.15
4	88.35	6.40	94.75	69.55	7.60	77.15	66.35	4.95	71.30
5	85.20	6.00	91.20	77.15	4.65	80.80	62.20	8.00	70.20
10	88.60	5.90	94.50	64.55	6.45	71.00	60.80	5.10	65.90
0 (Check 3)	86.90	5.75	92.65	67.75	8.60	76.35	65.80	4.10	69.90
20	85.70	3.75	89.45	68.75	6.90	75.65	79.00	3.00	82.00
30	76.95	3.00	79.95	55.00	3.85	58.85	74.00	3.55	77.55
50	67.95	3.55	71.50	25.35	1.40	26.75	45.70	2.00	47.70
0 (Check 4)	65.15	7.65	72.80	60.35	6.00	66.35	66.85	6.55	73.40
100	43.75	3.00	46.75	2.00	1.00	3.00	1.25	0.50	1.75
200	22.50	1.25	23.75	None	0.125	0.125	None	None	None
400	4.00	0.50	4.50	None	None	None	None	None	None

than the borax. Indeed, if the yield of "primes" is considered rather than the total yield, it is found that the plot which received 50 pounds of borax yielded 2.8 pounds more than the adjoining check plot.

With an application of 100 pounds of borax per acre the yield is cut to approximately one-half the normal and with 200 pounds of borax to about one-quarter the normal. With the 400-pound application, the crop was practically a failure, being less than one-twentieth of the normal.

Section II

For this section there is no distinct depression in yield that can be attributed to the borax until the 30-pound application of borax is reached, the total yield with 20 pounds being within 0.7 pound of the yield on check plot 3

which adjoins this plot. The 30-pound application caused a drop in yield and the 50-pound quantity brought it down to about one-third of the normal. With the 100-pound application, the total yield of potatoes was only 3 pounds. The still heavier applications resulted in complete failure.

The low yields on plot 1 of this section must be attributed to some other cause than the borax.

Section III

The highest total yield for this section—82 pounds—was from the plot which received 20 pounds of borax and the next highest—77.5 pounds—from the plot which received 30 pounds of borax per acre. Check plot 4 which is separated from the plot receiving 30 pounds of borax by only one plot (2 rows) gave a total yield of 73.4 pounds. The 30-pound application of borax, therefore, cannot be said to have caused any depression in the yield. The 50-pound application brought the "primes" down to 45.7 pounds, the total yield for this plot being 47.7. The 100-pound application gave a total yield of less than 3 pounds and the 200 and 400-pound applications resulted in total failure.

The heavy applications of borax either prevented germination entirely or resulted in a long delay in germination. On the plots which received these heavy applications a few plants came through but were several weeks later than the plants on the plots not affected by the borax. These delayed plants grew slowly, were slender and lacked vigor, and were abnormal in color. In some respects the injury is similar to the injury caused by soils heavily charged with alkalies.

THE CORN CROP

The same general plan was followed for the corn as for the potatoes. However, each plot or treatment consisted of a single row instead of two rows, as in the case of the potatoes.

The fertilizer was used at the rate of 400 pounds per acre, but the amount of borax applied remained the same as for the potatoes.

The fertilizer for section 1 was drilled in the furrow by hand on April 30. On May 15 the fertilizer was applied to sections 2 and 3 in the same manner as in the case of the potatoes, and the corn was planted on the three sections. The rows were run 4.5 by 3.4 feet. For each section there were 14 hills to the row with five kernels to the hill.

In order that additional information might be secured with reference to the influence of the borax on the germination of the corn, three single kernels were dropped (at equal distances from one another) between the hills.

On June 3 a count was made of the stalks in the hill and also for the inter-hill planting. The results of this count are shown in table 2. From this table it will be noted that on sections 2 and 3 germination was somewhat depressed with as low as 5 and 10 pounds of borax per acre. On section 1 this depression is noted at 20 pounds per acre for the hill planting and at

30 pounds for the inter-hill planting. With 50 pounds of borax per acre on section 2 only 9 out of a possible 70 plants were found for the hill planting, and only one out of a possible 42 for the inter-hill planting.

The inter-hill plants were finally removed and the hills thinned to 3 plants (some hills had only 2 plants).

TABLE 2

Germination count June 3; hill and inter-hill planting, corn borax experiment

HILLS	HILL PLANTING			INTER-HILL PLANTING		
	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3
0 (Check 1)	55	52	57	32	34	34
1	58	55	58	30	25	34
2	60	54	57	30	30	33
3	59	55	55	31	31	33
0 (Check 2)	56	54	54	26	31	27
4	50	45	54	27	27	26
5	44	42	49	28	18	31
10	52	36	42	28	16	24
0 (Check 3)	51	61	59	31	35	33
20	43	23	35	30	5	21
30	38	13	28	25	7	12
50	46	9	8	27	1	4
0 (Check 4)	52	53	52	27	28	28
100	32	2	7	18	1	4
200	8	0	0	6	0	0
400	0	0	0	4	0	0

TABLE 3

Air-dry weights of corn in borax experiment

PLOT	GRAIN			COBS			STALKS		
	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Check 1	11.30	19.88	19.59	2.43	4.32	4.33	24.25	22.00	21.40
1	7.96	16.79	17.39	1.63	3.73	3.44	25.00	26.95	18.90
2	8.87	14.93	20.53	1.81	3.18	4.33	23.00	25.75	23.60
3	14.30	13.36	16.79	3.05	2.69	3.44	21.15	18.20	20.80
Check 2	13.47	13.00	13.91	2.81	2.35	2.82	19.30	18.55	20.20
4	9.82	12.00	11.97	2.07	2.50	2.36	14.60	17.50	17.05
5	8.79	8.60	9.97	2.00	1.65	2.04	14.20	12.55	13.80
10	10.85	9.47	11.73	2.30	1.97	2.61	14.85	15.90	16.70
Check 3	7.95	11.68	15.90	1.83	2.44	3.16	18.70	16.52	19.85
20	8.64	9.24	13.90	1.93	1.78	2.84	18.10	15.13	21.05
30	11.77	7.57	12.63	2.67	1.99	2.86	22.95	16.70	21.70
50	11.08	3.41	6.79	2.62	0.92	1.66	18.75	7.30	15.65
Check 4	10.55	19.12	20.90	2.23	4.27	4.90	22.50	24.35	31.50
100	6.44	0.62	2.82	1.76	0.19	0.86	20.90	1.70	9.40
200	1.23	0.00	0.00	0.32	0.00	0.00	2.60	0.00	0.00
400	0.40	0.00	0.00	0.15	0.00	0.00	2.70	0.00	0.00

The corn received the usual cultivation during the summer and was harvested on October 11. The stalks and ears for each plot in the three sections were weighed and the weights of the stalks at this time were taken as the final weights. The ears, however, were dried and the corn shelled so that the weights here reported are for the dry shelled corn. The weights of stalks and corn for the three sections are shown in table 3.

Section I

The weights for section 1 do not indicate any definite depression in yield up to and including 50 pounds of borax per acre, this plot having yielded about ½ pound more than check plot 4 which adjoins it. With 100 pounds of borax and over the depression in yield is very pronounced. This is clearly indicated by figure 1, plate 4.

The low yields for the check plots of this section are apparently due to unfavorable soil conditions. The weights of the corn stalks for these check plots as compared with the corresponding weights for sections 2 and 3 would indicate that the stalk growth was about normal.

Section II

The yields on plots 1, 2, and 3 are less than the yield of check plot 1 but are more than the yield on check plot 2. It would, therefore, appear that the depression noted (as compared with check 1) is due to a soil condition rather than to the borax treatment.

Beginning with the 5-pound application there appears to be some depression in yield, though it may be pointed out that the yield was almost as much where 20 pounds of borax was used as where only 10 pounds was used. With 50 pounds of borax the yield was reduced to 3.41 pounds and with 100 pounds to 0.62 pounds, as compared with 19.2 pounds for the adjoining check. With the 200 and 400-pound applications the crop was a complete failure.

The appearance of section 2, including plots 20, 30, 50 and check 4 on July 6, is well illustrated by figure 2, plate 4. It will be noted that the few stalks which did survive, were gradually recovering from the set-back given by the borax.

Section III

For this section there is not positive indication of injury until the 4-pound application is reached and possibly not until the 5-pound application. Indeed what appears to be a depression with 5 pounds may be due to a soil condition since the yield with 10, 20 and 30 pounds is considerably higher than the yield with 5 pounds.

For the 50-pound application the yield was 6.79 pounds, whereas the yield on the adjoining check plot was 20.9 pounds. The 100-pound application gave a yield of 2.82 pounds. The larger applications resulted in complete failure.

RAINFALL RECORD

It is well known that the rainfall during any given season has an important bearing on crop yields, and that the effectiveness of fertilizer salts may vary widely depending upon whether there is much or little rain. The same would apply to other soluble salts as, for example, borax.

The rainfall at New Brunswick during the summer of 1920 was unusually heavy, as shown by the monthly record in comparison with the average for the same months during the last ten years. The monthly rainfall from April to September, inclusive, 1920, and the 10-year average—1910 to 1919—for the same months, is shown in table 4.

In connection with the effect of the borax on the potatoes it may be pointed out that between April 16 and 27—the time that the fertilizer remained in the ground on section 1, preceding the planting of the potatoes—the rainfall was 2.01 inches. On the day following the planting of the potatoes there was a fall of 0.6 inch.

TABLE 4

Rainfall at New Brunswick, New Jersey

	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER
	inches	*inches*	*inches*	*inches*	*inches*	*inches*
1920	4.28	3.56	9.64	6.00	8.21	2.23
10-year average, 1910–1919	3.66	3.85	3.52	4.67	5.07	2.95

It is not probable that a rainfall of 2 inches in 2 weeks, fairly distributed, would wash very much of the borax entirely out of reach of the potato tubers. It would, however, result in more or less diffusion of the borax through the soil, and this diffusion, together with a certain amount of colloid absorption, could no doubt, account for the smaller amount of injury on section 1, where the fertilizer was in the ground 2 weeks before the potatoes were planted, than on the other two sections.

Between April 30, when the fertilizer was applied to section 1 for corn, and May 15, when the corn was planted, there was a rainfall of 2.33 inches, fairly distributed over the period. As in the case of the potatoes this resulted in more or less diffusion of the borax through the soil with the result that germination was more nearly normal on this section than on sections 2 and 3, where the corn was planted at the time of applying the fertilizer.

Had the rainfall for the season been normal or below normal, instead of considerably above normal, it is possible that the 5, 10, 20, 30 and 50-pound applications of borax might have resulted in greater injury to both corn and potatoes.

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PLATE 1

Fig. 1. Comparing 5, 10 and 20 pounds of borax with check 3

Fig. 2. Comparing 400, 200, and 100 pounds of borax on sections 1, 2 and 3

FIG. 1

FIG. 2

PLATE 2

FIG. 1. Comparing the yield of potatoes on check 3 with 30 pounds of borax on sections 1, 2 and 3.

FIG. 2. Comparing the yield of potatoes on check 4 with 50 pounds of borax on sections 1, 2 and 3.

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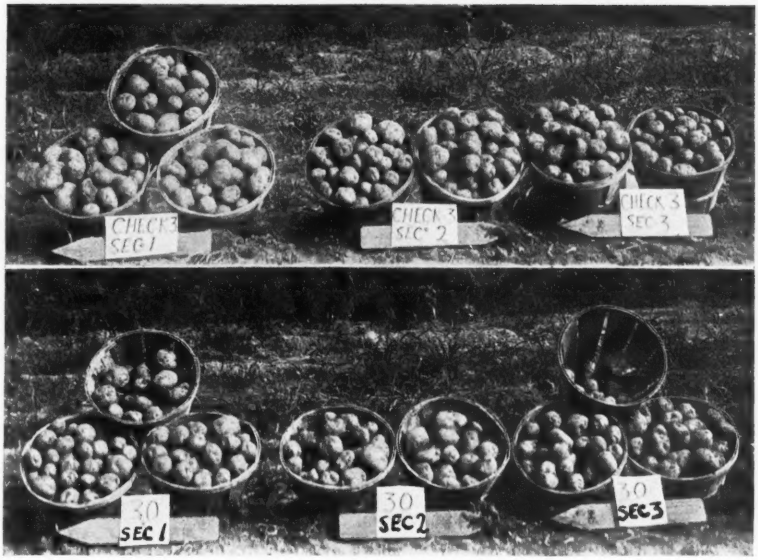

FIG. 1

FIG. 2

PLATE 3

FIG. 1. Comparing the yield of potatoes with 10 and 20 pounds of borax on sections 1, 2 and 3.

FIG. 2. Showing appearance of residual crop of rye where 100, 200 and 400 pounds of borax were used; no apparent injury to date.

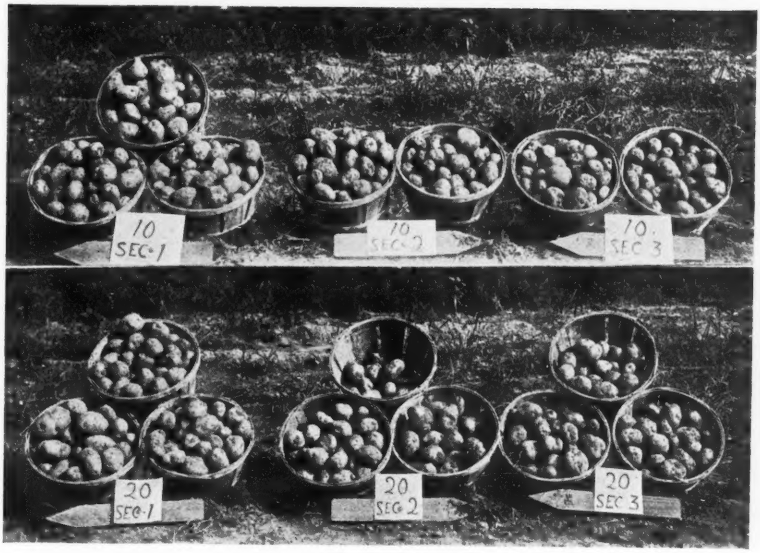
10
SEC 1
10
SEC 2
10
SEC 3
20
SEC 1
20
SEC 2
20
SEC 3

FIG. 1


100
200
NOV. 4 1920
400

FIG. 2

PLATE 4

FIG. 1. Showing corn on section 1 where 100, 200 and 400 pounds of borax were used, as compared with check 4.

FIG. 2. Showing corn on section 2, where 20, 30, and 50 pounds of borax were used, as compared with check 4.

FIG. 1

FIG. 2

SULFUR FOR NEUTRALIZING ALKALI SOIL[1]

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Among numerous experiments with alkali soils made by the writer, some, in which sulfur was the active agent, seem of sufficient importance to be worthy of public notice.

About a year ago 1 per cent of sulfur was added to an alkali soil; to another portion of soil was added 1 per cent sulfur + 1 per cent alfalfa meal; to another portion 1 per cent sulfur + 1 per cent pulverized manure. With these was a jar of the soil without any treatment. Water was added to all four to bring the moisture content to 15 to 18 per cent. The jars of soil were covered loosely and kept in a storeroom for several months without attention. When examined, the three jars to which sulfur was added were found to be quite acid. The sulfur had been oxidized to sulfuric acid which had neutralized the sodium carbonate of the soil. The soil in the jar to which no sulfur was added was as alkaline as at first.

A second experiment was soon started to study the matter in more detail. The total alkalinity of a number of soils was determined by titration of a suspension in water, the theoretical amount of sulfur then added and water to bring to optimum for plant growth. From time to time the soils were tested. After 10 days it was found that from 50 to 90 per cent of the original alkalinity had been neutralized in all but two. These were very alkaline and saline at the start. Two fertile non-alkaline soils had become acid though only 0.02 per cent sulfur had been added to them (table 1). After some months the alkalinity of the alkali soils was still further reduced, but none of them became acid. The slight residual alkalinity was probably chiefly due to calcium carbonate.

For the third experiment there were taken 6.5 kgm. of an alkali soil which had been washed so that most of the soluble salts and a large portion of the alkalinity had been removed. In this soil barley germinated poorly and grew very little. A number of other plants likewise failed. Only Bermuda grass seemed to do well. The soil was dried, pulverized and mixed with the theoretical amount of sulfur to neutralize its remaining alkalinity. The sulfur used was ordinary flowers of sulfur. After two weeks most of the alkalinity had

[1] Since this article was written, the attention of the writer has been called to a note by J. G. Lipman in which the use of sulfur on alkali soil was suggested. It was dated August 24, 1916, and published in SOIL SCIENCE, 1916, v. 2, p. 205.

disappeared and there was a notable increase in soluble SO_4. Twelve barley seeds were planted. A week later all the seeds had sent up sprouts, some 2 inches high. All but three were removed. These three are still growing (2 months old) and apparently fairly healthy, 10 to 15 inches high. However, their growth is much slower than would be expected in a fertile soil. The slow growth may be accounted for by the lack of available plant-food consequent to the considerable washing which the soil had received. But there was little evidence of the toxic alkalinity which had prevented the growth of barley before the treatment with sulfur.

TABLE 1

Reduction of alkalinity following additions of sulfur to alkali soil

SOIL NUMBER	SULFUR ADDED	pH	ALKALINITY OF SOIL EXPRESSED AS PER CENT H_2SO_4 NECESSARY TO NEUTRALIZE AT VARIOUS DATES				
			Beginning June 18	June 29	July 15	August 30	December 10
	per cent						
16	0.33	9.0+	1.00	0.70	0.70	0.54	0.30
17	0.10	9.0+	0.33	0.17	0.17	0.07	0.07
18	0.02	7.0	0.01	0.01	0.01	0.01	0.01
19	0.03	8.0	0.09	0.05	0.02	0.02	0.02
20	0.05	8.5	0.16	0.08	0.03	0.02	0.02
A	0.05	8.5	0.14	0.07	0.02	0.02	0.02
B	0.09	8.8	0.26	0.08	0.05	0.03	0.02
C	0.06	8.2	0.18	0.06	0.05	0.01	0.01
D	0.03	8.2	0.10	0.03	0.02	0.01	0.01
15	0.02	6.7	0.00	Acid			
1c	0.02	6.8	0.00	Acid			

All these soils except 1c are sandy loams. Soil 1c is a fertile clay loam; no. 15 is a fertile sandy loam.

Soils 16 and 17 have high alkalinity and high salts, no. 18 has high salts, and no alkalinity; nos. 19 and 20 have moderate salts and alkalinity. Soils A and B were produced by washing soil 17 until most of the salts were removed. Soil C is similarly derived from no. 20 and D from no. 19 by washing.

In another experiment a neutral soil was produced by mixing calculated amounts of an alkali soil with the proper amount of an acid soil which had been made by treatment with an excess of sulfur.

From these experiments it is inferred that sulfur added to a soil soon becomes sulfuric acid which reacts with and neutralizes whatever alkaline material may be present. This effect should be of great value in the reclamation of alkali land. To those who have studied the reclamation of alkali land it is common knowledge that it is very difficult to remove the last of the alkalinity by leaching. Instead of trying to remove all the alkalinity by leaching it would seem more practicable to neutralize it by the addition of sulfur after most of the salts had been removed by water. In this way the soil would not be so impoverished of available plant-food as by the long leaching which would

otherwise be necessary to remove the last of the hydrolyzable salts causing toxic alkalinity.

It seems probable that oxidation of sulfur in soil is largely due to biologic action. Assuming this to be true, it is likely that such oxidation would not take place in a soil which was too alkaline for active bacterial growth. In two such soils, 16 and 17, which will not support any ordinary plant, sulfur is very slowly oxidized, and much of the original high alkalinity yet remains. However, it would be unnecessarily expensive and usually prohibitive to neutralize all the alkalinity of such soils with sulfur. Instead, most of the alkaline matter and salts should be leached out with water, then sulfur applied to neutralize the residual alkalinity which is difficult to wash out.

THE EFFECT OF ORGANIC NITROGENOUS COMPOUNDS ON THE NITRATE-FORMING ORGANISM[1]

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The behavior of the nitrifying bacteria in the presence of organic substances is a matter of special interest to the bacteriologist. It was one of the problems to which much time and energy was devoted by the Franklands, Warington, Winogradsky, Omelianski, Fremlin, Beijerinck and others. According to the results of most of these investigators soluble organic substances, especially if present in large amounts, are injurious to the nitrate-forming organism. Unfortunately the data agree neither in respect to the nature of the injury nor to the kind and amount of substances required to produce this injury. Perhaps this variation was due to the fact that in many cases the cultures under study were not pure. A review of the literature on the effect of organic substances on the nitrifying bacteria shows clearly the variation in results obtained by different investigators.

Winogradsky (8, 9) in his earlier work says that nitrifying bacteria do not require any organic substance for their growth and that even the slightest trace is toxic to them. There is every reason to suppose that the process of nitrification, which goes on in the soil in the presence of organic matter, ought not to be so sensitive to small amounts of this material in a synthetic medium. Beginning with Winogradsky, most of the investigators have reported that nitrifying bacteria do not grow in bouillon.

The Franklands (3) reported that the nitrifying organism in broth produced very characteristic growth, slow in commencing, but luxuriant; and that nitrification took place in an ammoniacal solution inoculated from such broth cultures. They found that microscopically its form differed slightly when grown in broth and in ammoniacal solutions, yet returned to its characteristic bacillo-coccus form when grown again in an ammoniacal solution. They also grew it in peptone gelatin by passing it first through broth cultures.

Warington (6) used an impure culture of the nitrous organism to seed solutions of ammonium carbonate containing 20 per cent, 5 per cent, and 1 per cent of broth. All of these solutions nitrified; they were always turbid. Stained preparations showed that the bacilli diminished in number as the proportion of broth decreased, while the deeply stained minute dots became

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

more manifest. He reported that the pure nitrous organism grew slowly in weak broth, but without impairing its transparency or producing any other visible change; and that it was capable of producing nitrous acid in dilute solutions of asparagin, milk, urine and urea. All of these cultures were free from turbidity. In pure cultures of the nitrous organism in ammoniacal solutions, the addition of carbonic acid, sodium bicarbonate or calcium acetate facilitated nitrification. A pure culture would not ordinarily grow on gelatin; if it did, it lost its power of oxidation.

Fremlin (4) reported that the nitroso-bacterium would grow not only in silica jelly but also in any ordinary organic medium. He maintained that there were not two separate and distinct species of this organism; the one able to convert ammonia into nitrous acid and capable of being cultivated only in special media, the other able to grow on ordinary media with no ability to convert ammonia into nitrous acid.

Burri and Stutzer (2) reported the isolation of a nitrate-forming organism which gave a well-defined growth in the presence of organic substances which are commonly used in bacteriological culture media. Attention was called to the loss of the oxidizing power of cultures which developed in organic solutions. Examination of their culture by Winogradsky (10) showed the presence of four organisms, one of which proved to be *nitrobacter*. Since their results were obtained with an impure culture the report is not conclusive.

Winogradsky and Omelianski (11) and later Omelianski (5) alone carried out a very careful and extensive study of the influence of organic substances on the nitrate-forming bacteria. Cultures of the nitrate-forming organism from two sources, one from Germany and one from St. Petersburg, were used. Although these cultures were from widely separated sources geographically, they did not show any difference in physiological characteristics. These organisms failed to produce any turbidity in bouillon and gave a rapid oxidation of nitrite to nitrate. The following table gives the smallest concentration of the organic substance which caused a noticeable decrease in nitrate formation and the concentration which prevented it.

COMPOUND	RETARDS	PREVENTS
	per cent	*per cent*
Glucose	0.05	0.2–0.3
Peptone	0.80	1.25
Asparagin	0.05	0.5–1.0
Glycerin	0.05	1.00
Urea	0.50	1.00
Sodium acetate	1.50	3.00
Sodium butyrate	0.50	1.00
Beef broth or infusion	10.00	60.00

They found that the injurious effect of organic substances depended upon the composition and amount of organic substance, and also on the amount of

the inoculum. In other words, a large number of the nitrate organisms when transferred to a medium containing organic substances could withstand more organic matter than a small number. By repeated transfers to solutions containing increasing amounts of bouillon, the nitrate organism became adapted to 50 per cent of bouillon. Examination of the unoxidized cultures containing organic substances showed that the organisms were killed. In other words, failure to oxidize was due to injury of the cells rather than to growth of the nitrate ferment accompanied by a loss of its power of oxidation.

Beijerinck (1) reported the growth of pure cultures of the nitrate-forming organism in the presence of various kinds of organic substances. The chief points of interest in the paper are that growth and oxidation are two separate functions; that growth in the presence of soluble organic substances causes the nitrate-forming organism to lose its power of oxidation. He found that small amounts of the organic substance, less than 0.05 per cent of glucose, sucrose, starch, mannitol, sodium and calcium acetate, peptone, tyrosin and asparagin did not prevent the oxidation of nitrite to nitrate. He concluded that reproduction of the organisms in solutions containing appreciable amounts of organic substances caused a stable modification of their physiology. Moreover, all attempts to secure oxidation from cultures which had grown in organic solutions failed.

EXPERIMENTAL

The work reported in this paper was prompted in part by the contradictory results of previous investigators on the effect of organic substances on the growth and oxidizing power of the nitrifying organisms. It was noted in our preliminary work on nitrification that, when *nitrobacter* cultures were being tested for purity in Nährstoff-Heyden solution, contact with this organic medium did not prevent the organisms from oxidizing nitrite to nitrate when they were transferred from it to a nitrifiable medium. Because of this additional evidence, experiments were planned to include the effect of several other organic substances on the nitrate former.

For this work cultures of the nitrate-forming organisms were obtained by seeding shallow layers of liquid nitrite medium with 1-gm. portions of fresh soil. The medium was a modification of that used by Omelianski (5) and was prepared as follows:

Sodium nitrite ($NaNO_2$)	1.0 gm.
Dibasic potassium phosphate (K_2HPO_4)	0.5 gm.
Sodium chloride (NaCl)	0.5 gm.
Sodium carbonate (Na_2CO_3) anhydrous	0.5 gm.
Magnesium sulfate ($MgSO_4.7H_2O$)	0.3 gm.
Ferrous sulfate ($FeSO_4.7H_2O$)	Trace
Water distilled	1000.0 cc.

After 1 to 2 weeks at 28°C. the nitrites were oxidized to nitrates and subcultures were made in flasks of fresh nitrite medium. In this way the nitrate

producers were carried through 50 sub-cultures, and to the fiftieth transfer, a fresh dose of a sterilized solution of sodium nitrite (0.01 gm. $NaNO_2$ to 10 cc. of liquid culture) was added. This procedure of adding a dose of nitrite to the cultures as soon as they were oxidized was repeated eleven times, and these cultures are designated as enrichment cultures. The cultures were obtained from different soils; those from field soil are numbered 1, 1^1, and 1^2, and those from garden soil are numbered 2, 2^1, and 2^2.

Purity tests of enrichment cultures, in peptone-beef infusion and in Nährstoff-Heyden solution

An attempt was made to purify cultures 1, 1^1 and 1^2 and 2, 2^1 and 2^2 by subjecting them to as many oxidations of sodium nitrite as could be brought about in the same culture. At the end of each oxidation of nitrite to nitrate in these enrichment cultures, the ability of these cultures to give visible growth in either peptone-beef infusion or in Nährstoff-Heyden solution was tested by inoculating these two media with the oxidized cultures. Two tubes of each medium were used for this test, one tube being inoculated with a loopful, and one tube with 0.5 cc. of the culture to be tested.

The term "peptone-beef infusion" is used to designate a medium prepared according to the following formula: 500 gm. of fresh lean beef, 10 gm. of peptone, and water to make 1 liter. The "peptone-beef extract" consisted of beef extract (Liebig's) 3 gm., peptone 10 gm., and 1000 cc. of water. The reaction in both media was adjusted to pH 7.0 to 7.6. The Nährstoff-Heyden solution was made by heating 7.5 gm. of the Nährstoff-Heyden powder with 1 liter of distilled water for 1 to 2 hours in a steamer; the filtered solution was used without any change in its reaction, which in terms of hydrogen-ion concentration was pH 7.8. Determination of growth in these tubes was made after an incubation of 2 weeks at 28°C. The controls were uninoculated flasks of the medium, which received an addition of sterilized sodium nitrite at the same time as the cultures, and which were tested in peptone-beef infusion or in Nährstoff-Heyden solution in exactly the same manner as were the cultures. The introduction of Nährstoff-Heyden solution as a test for the purity of *nitrobacter* cultures was the result of the use of this medium for the growth of soil organisms, where it was found that it supports the growth of many more soil types than does peptone-beef infusion.

The purity tests of the enrichment cultures did not give the wholly consistent results which might be expected, namely, that growth in peptone-beef infusion would become less after each oxidation of added nitrite. But many factors might have been the cause of the irregularities with reference to growth in peptone-beef infusion: the possibilities of contamination because of the long period of time the enrichment cultures were incubated without any special protection from the dust of the air, and the possibilities of contamination when the cultures were opened for the addition of nitrite and the removal of

inocula for the purity tests, were considerable, as was shown by the fact that a number of controls became contaminated during the experiment.

However, at the end of the sixth oxidation in each of these cultures it was evident that the cultures were gradually becoming purified, since all of them gave doubtful growth in peptone-beef infusion. As compared with peptone-beef infusion a decided change was seen in the growth of these cultures in Nährstoff-Heyden solution. The seventh and eighth oxidations of the same cultures immediately following the peptone-beef infusion tests showed growth in the case of all the cultures, and this after rather doubtful growths in peptone-beef infusion. It was plainly noticeable that where all cultures showed profuse growth in Nährstoff-Heyden at the end of the seventh oxidation, at the end of the ninth oxidation, this growth was much less evident. From the results of additional experiments, it was found that after several oxidations of sodium nitrite in a culture, such a culture when inoculated into Nährstoff-Heyden solution, gave much less noticeable growth than did the same culture in which only a few oxidations had taken place.

Isolation of pure cultures of nitrobacter

In none of the tests in Nährstoff-Heyden solution did any of these enrichment cultures give negative results in both the loop and the 0.5-cc. inoculations. In addition to the usual test adopted for purity of *nitrobacter* cultures, i.e., failure to cause visible growth in alkaline peptone-beef infusion (see Wimmer (7)) it was thought desirable to apply a more critical test, and to study only such cultures as failed to give growth, after 2 weeks' incubation at 28°C., in Nährstoff-Heyden solution, when inoculated either with a loopful or 0.5 cc. of the *nitrobacter* culture.

With this test in mind, dilutions of the cultures used in the preceding experiments were made in sterilized sand water blanks. Flasks of sodium nitrite liquid medium, plates of nitrite agar, and tubes of Nährstoff-Heyden solution were inoculated from each dilution. All were incubated at 28°C. The nitrite agar was made by dissolving the salts, as given previously, in a liter of a 1.5 per cent solution of washed agar. The hydrogen-ion concentration of the resulting medium was between pH 8.8 and 9.0.

When the flasks of nitrite solution inoculated from these dilutions, after 5 weeks' incubation, were tested for the presence of nitrates and the absence of nitrites, it was found that oxidation had taken place up to and through the fourth dilution (1 in 400,000), of culture 1 from field soil, and the fifth dilution (1 in 10,000,000) of culture 2 from garden soil. Positive growth was given in Nährstoff-Heyden solution only in the tubes inoculated from the first dilution. The plates were examined after 7 weeks' incubation. The colonies in the first and second dilutions all had about the same appearance. These plates were heavily seeded with tiny white-appearing colonies; the smallest could be seen only by holding the plates up to the light and looking through them. Growth

was cut down in the third and fourth dilutions and only an occasional colony was found in plates above these dilutions.

When 8 weeks old the cultures on nitrite agar were examined again. All the plates of the lower dilutions showed numerous small colonies, cream to brown in color and often deep in the agar. Slopes of nitrite agar were inoculated from four different colonies: 1^3 a brown colony which, when stained, looked like *nitrobacter;* 1^4 a large spreading brownish colony; 1^5 a large spreading whitish colony; and 1^6 a small brown colony. After 17 days at 28°C. transfers were made in triplicate from these tube cultures into liquid sodium nitrite medium; and 10 days after inoculation two of the 1^3 cultures were oxidized, and in 14 days a third of the 1^3 cultures was oxidized. These were all transferred to sterile liquid nitrite medium and tested for purity in Nährstoff-Heyden solution; none gave visible growth in Nährstoff-Heyden solution. Cultures 1^4, 1^5 and 1^6 failed to oxidize the nitrite to nitrate. In a similar manner cultures 1^7 from field soil and 2^3 from garden soil were secured. Here again oxidation took place and no turbidity in peptone-beef infusion or in Nährstoff-Heyden solution was noted.

Growth or existence of nitrobacter in the presence of organic nitrogenous substances

Under field conditions the presence of organic compounds of nitrogen favors in a marked degree the process of nitrification. According to the reports of many investigators, such substances are reported as having an injurious effect when added to a medium for the growth of the organisms concerned in the process. Such opposite effects hardly seem plausible and the subject deserves further study. Only a few of the possibilities along this line of investigation have been taken up in this paper.

Beef infusion. Experiments to test the toxicity of beef infusion toward *nitrobacter* were made in which 3 strains of the organism were inoculated into a fresh meat infusion (500 gm. of meat and 1000 cc. of H_2O, pH 7.6). After 2 weeks in this medium and no apparent growth, loop and 0.5-cc. portions were seeded into nitrite solutions. At regular intervals of 2 days each the nitrite solutions were tested for nitrates. Although these cultures were kept for 6 weeks no oxidation was ever noted in any of them. The data indicate the poisonous effect of beef infusion on the *nitrobacter* organism.

Peptone-beef extract and Nährstoff-Heyden. In a second test beef infusion was replaced with peptone-beef infusion and for comparison Nährstoff-Heyden solution also was used. These two nitrogenous culture solutions were seeded with *nitrobacter* cultures 1^3 and 2^3, and incubated for 14 days. No visible evidence that growth had taken place, i.e., clouding of the media, was ever noted. One-half cubic centimeter portions of these 14-day-old cultures from peptone-beef infusion and from Nährstoff-Heyden were added to flasks of the nitrite medium. The results of oxidation tests are given below:

I. From Nährstoff-Heyden:
  Culture 1^7—One oxidized in 17 days
  One oxidized in 13 days.
  Culture 2^3—One oxidized in 20 days
  One oxidized in 29 days.
II. From Peptone-Beef infusion:
  Cultures 1^7 and 2^3, neither oxidized.

A repetition of this experiment later confirmed the results that peptone-beef infusion is harmful and Nährstoff-Heyden, non-toxic to the *nitrobacter* organism.

In a third set of experiments, cultures 2 weeks old of different strains of *nitrobacter* 1^3 and 1^7 from nitrite agar slants were transferred to tubes of peptone-beef extract, Nährstoff-Heyden solution, urine, and sterilized water. Immediately after inoculation and each day thereafter, for 1 week, microscopical preparations were made from all of the cultures. These stained mounts were examined carefully, but failed to show any conclusive evidence that the organisms had reproduced. None of the tube cultures gave any evidence of clouding or turbidity. From this evidence it seems that *nitrobacter* does not reproduce in water, urine, peptone-beef extract, or Nährstoff-Heyden solution; although there is possibly a slight increase in the last-named medium.

After 12 days in these various media, transfers were made to small flasks of liquid nitrite medium. The results of these qualitative tests are given below:

1. From Nährstoff-Heyden:
   Cultures 1^3 and 1^7 both oxidized in 9 days.
2. From Peptone-Beef extract:
   Cultures 1^3 and 1^7, neither oxidized.
3. From Urine:
   Cultures 1^3 and 1^7, neither oxidized.
4. From Water:
   Cultures 1^3 and 1^7 both oxidized in 18 days.

The nitrogenous substances peptone-beef extract and urine, either killed the bacteria or destroyed their power of oxidation; while, on the other hand, Nährstoff-Heyden did not exert a harmful effect, since these cultures oxidized faster than those from water.

From the results of the preceding test it is shown that *nitrobacter* will live, perhaps reproduce, in the presence of certain organic compounds without any loss of its power to oxidize nitrites. *Nitrobacter* can be cultivated on agar which contains Nährstoff-Heyden. Experiments have been carried out to test the behavior of this organism on Nährstoff-Heyden agar slants, with and without nitrite present. It was found that pure cultures of *nitrobacter* made a fair growth on Nährstoff-Heyden agar, but a better growth when sodium nitrite was added to the Nährstoff-Heyden. When this growth on the Nährstoff-Heyden agar slants or on nitrite agar slants containing Nähr-

stoff-Heyden was transferred to liquid cultures of the nitrite medium, oxidation resulted in about 6 to 8 days. The time required for oxidation was less than that noted in the case of flasks inoculated from washed sodium nitrite agar cultures. The beneficial effect of the Nährstoff-Heyden is clearly indicated from these results.

Effect of dilution on the harmful substance found in beef infusion

To measure the degree of toxicity of fresh beef infusion, it was prepared according to the following method: 500 gm. of fresh lean beef was extracted for 4 hours at 55°C. with 1000 cc. of water. The liquid infusion was then

TABLE 1

Effect of dilution of beef infusion on the injurious factor

NUMBER	AFTER 7 DAYS IN	REACTION		
		pH	Culture No. 1[8]	Culture No. 1[7]
1	Sterilized water		All oxidized	All oxidized
2	Meat infusion	7.0	No oxidation	No oxidation
3	Meat infusion diluted 1–1 with water	6.9	All oxidized	All oxidized
4	Meat infusion diluted 1–2 with water	6.9	All oxidized	All oxidized
5	Meat infusion diluted 1–4 with water	7.0	All oxidized	All oxidized
6	Meat infusion diluted 1–10 with water	7.0	All oxidized	All oxidized

TABLE 2

Source of the injurious substance or substances found in beef infusion

NUMBER	AFTER 7 DAYS IN	REACTION	TIME REQUIRED FOR THE OXIDATION OF NITRITES TO NITRATES	
			Culture 1[8]	Culture 1[7]
		pH	*days*	*days*
1	Sterilized water		7	7
2	Meat infusion sterilized for 30 minutes at 15 pounds pressure	7.0	No oxidation	No oxidation
3	Volatile distillate from meat infusion at 100°C	8.2	26	14
4	Residue from steam distillate	6.2	No oxidation	No oxidation
5	Ether extract of meat infusion	5.2	No oxidation	No oxidation
6	Residue from ether extract	6.5	No oxidation	20
7	Alcoholic extract of meat infusion (70 per cent alcohol)	6.4	No oxidation	No oxidation
8	Residue from alcoholic extract	7.0	16	18

filtered from the beef and its reaction made to pH 7.8–8.0 after which it was boiled for ½ hour, made up to volume, filtered and sterilized. This beef infusion was diluted with water in varying amounts and inoculated with *nitrobacter*. After 1 week, transfers were made to liquid nitrite medium. The results are given in table 1. Here it will be seen that beef infusion is

non-toxic when diluted 1 to 1 with water. The harmful substance, therefore, is present in relatively small amounts or it is only a weak poison.

Table 2 gives the results of a second experiment. If the beef infusion is subjected to a steam distillation, the harmful agent remains in the residue while the distillate is entirely free of any poison. The ether and alcoholic extracts of the beef infusion contained this harmful substance while the residues remaining after extraction with ether and alcohol were in all cases decidedly less toxic and in some cases not toxic at all. From the figures of this table it will be seen that the ether and alcoholic extracts are more acid than the beef infusion. It was thought that possibly this acid reaction was concerned with the injurious agent. New extracts were prepared and adjusted to a pH 8.0. Here again in an alkaline reaction these extracts injured the nitrate organism.

The effect of different nitrogenous organic substances on oxidation

The results from beef infusion and Nährstoff-Heyden solution with the nitrate-forming organism suggested the use of a wider variety of organic nitrogenous compounds. Accordingly, three *nitrobacter* cultures, 1^3, 1^4 and 2^3, supposedly pure, i.e., no turbidity in peptone-beef infusion or Nährstoff-Heyden solution, were inoculated in duplicate in loopful and 0.5-cc. portions into the following media:

(1) Nährstoff-Heyden 1 per cent, reaction.......................... pH 7.4–8.0
(2) Gelatin 1 per cent, reaction.................................. pH 6.8–8.0
(3) Peptone 1 per cent, reaction.................................. pH 7.4–8.0
(4) Casein 1 per cent, reaction................................... pH 7.4–9.0
(5) Yeast water (28 gm. pressed yeast in 500 cc. of water), reaction. pH 6.8–8.0
(6) Skimmed milk, reaction.. pH 6.4–6.8
(7) Beef extract 1 per cent, reaction............................. pH 7.2–9.8
(8) Sterilized water (distilled)

To be sure that the cultures used in this experiment contained active oxidizing organisms, transfers were made to small flasks of liquid sodium nitrite medium at the same time that the organic media were inoculated. All three cultures oxidized the nitrite completely to nitrate.

Two weeks after the various organic media had been inoculated with the *nitrobacter* cultures, flasks of nitrite media were seeded from them, 0.5-cc. portions were used as inocula. The results are given below:

1. From Nährstoff-Heyden, all cultures oxidized in 6 to 8 days.
2. From gelatin, all cultures oxidized in 6 to 9 days.
3. From peptone, cultures 1^3, 1^7 and 2^3 oxidized in 8 to 12 days.
4. From casein, cultures 1^3, 1^7 and 2^3 oxidized in 8 to 12 days.
5. From yeast water, cultures 1^3, 1^7 and 2^3 oxidized in 12 to 15 days.
6. From skimmed milk, all cultures oxidized in 14 to 17 days.
7. From beef extract, none of the cultures oxidized.
8. From water, all cultures oxidized in 6 to 8 days.

When the same tubes of the different organic nitrogenous substances inoculated with cultures 1^3, 1^7 and 2^3 were 6 weeks old, their oxidizing power was again tested in sodium-nitrite liquid medium. The results follow:

1. From Nährstoff-Heyden, all cultures oxidized in 10 days.
2. From gelatin, all cultures oxidized in 19 days.
3. From peptone, all cultures oxidized in 16 days.
4. From casein, all cultures oxidized in 12 to 14 days.
5. From yeast water, all cultures oxidized in 14 days.
6. From skimmed milk, none of the cultures oxidized.
7. From beef extract, none of the cultures oxidized.
8. From water, all cultures oxidized in 10 days.

After 2 weeks in these various nitrogenous solutions all of the cultures retained their oxidizing power except those from beef extract. In the presence of this substance the nitrate producer either loses its power to oxidize or is killed. No visible growth was noted in any of the tubes of organic nitrogenous solutions. After 6 weeks in these various media, the results were, in general, similar to those obtained after the 2-week period. The milk cultures offer an exception in that no oxidation was observed. Casein, peptone, yeast water, and gelatin retarded somewhat the oxidizing power of *nitrobacter*.

As shown from the results of these two tests, the nitrate-forming organism will live for a period of 6 weeks, perhaps much longer in a medium rich in certain nitrogenous compounds without any loss of oxidizing power. The rate of oxidation in the cultures from Nährstoff-Heyden solution was just as fast as in the cultures from water, and in certain cultures more rapid.

Effect of organic nitrogenous substances on the oxidizing power of nitrobacter in enrichment cultures

The influence of organic nitrogenous substances on the oxidation of nitrite by *nitrobacter* was studied. Here the nitrogenous substance was added direct to the nitrite medium. The three cultures, 1^3, 1^7 and 2^3, which from previous tests failed to show visible growth in peptone-beef infusion or in Nährstoff-Heyden solution, were used. These nitrate formers were first inoculated into tubes of organic substances; one tube received 1 loop and another tube 0.5 cc. of the culture. After 2 weeks at 28°C. these tube cultures furnished the inocula for flasks of nitrite medium. At the time of inoculation and at the end of 2 weeks each tube culture was carefully examined by means of stained mounts, and only a few organisms were found in any of them.

The *nitrobacter* liquid cultures, the inocula of which were a 2-weeks-old suspension of *nitrobacter* organisms from one of the various organic nitrogenous substances, received, when oxidized, a second addition of 0.01 gm. of sodium nitrite. At the same time 1 cc. of a 1 per cent solution of the nitrogenous substance already present also was added to the flask cultures. The results of this second oxidation of nitrite in the presence of organic nitrogen, as well as

a third and fourth oxidation in the same culture flasks, are given in table 3. Oxidation progressed rapidly in all of the cultures except those inoculated from the beef-infusion tubes. The time required for oxidation varied according to the substance. Nährstoff-Heyden and gelatin gave the most rapid oxidation.

TABLE 3

Effect of organic nitrogenous substances on the oxidizing power of nitrobacter in enrichment cultures

NITROGENOUS SUBSTANCE ADDED	TIME REQUIRED FOR OXIDATION OF NITRITE TO NITRATE										
	Second addition of nitrite			Third addition of nitrite			FOURTH ADDITION OF NITRITE				
	4 days	5 days	7 days	3 days	5 days	12 days	2 days	3 days	4 days	6 days	10 days
1cc.											
Nährstoff-Heyden..	1^3 1^3			1^3 1^3			1^7 1^7	1^3	1^3		
	1^7 1^7			1^7 1^7							
	2^3 2^3			2^3 2^3				2^3 2^3			
Gelatin....	1^3 1^3			1^3		1^3	1^7 1^7	1^3			
	1^7 1^7			1^7 1^7					1^7	1^7	
	2^3 2^3			2^3 2^3				2^3 2^3			
Peptone....	1^3		1^3			2^3					
	1^7 1^7		2^3								
	2^3										
Casein.....	1^7 1^7	1^3 1^3			1^3 1^3			1^7 1^7	1^3	1^3	
				1^7 1^7							
	2^3		2^3		2^3 2^3					2^3	2^3
Yeast water	1^3 1^3			1^3				1^3			
	1^7 1^7	2^3 2^3									
	2^3				2^3						
Skimmed milk.....	1^3 1^3										
	1^7 1^7										
	2^3 2^3				2^3		2^3				

Effect of nitrogenous substances on the rate of nitrate formation

In this test 1 cc. of a 1 per cent aqueous solution of the various nitrogenous compounds was added at the time of inoculation to each 10 cc. of the sodium-nitrite medium. Only two cultures, 1^7 and 2^3 were used. As a control 1^7 and 2^3 were inoculated into the usual inorganic liquid medium containing sodium nitrite. The results are given below:

(1) Nährstoff-Heyden, cultures 1^7 and 2^3 oxidized in 5 days.
(2) Gelatin, culture 2^3 oxidized in 14 days and culture 1^7 in 18 days.
(3) Peptone, cultures 1^7 and 2^3 oxidized in 5 days.

(4) Casein, culture 2^3 oxidized in 6 days and culture 1^7 in 9 days.
(5) Skimmed milk, culture 1^7 oxidized in 5 days and culture 2^3 in 7 days.
(6) Beef extract, cultures 1^7 and 2^3 oxidized in 5 days.
(7) Beef infusion, culture 1^7 oxidized in 5 days and culture 2^3 in 7 days.
(8) Asparagin (0.5 per cent), culture 1^7 oxidized in 14 days and culture 2^3 in 18 days.
(9) Urea (0.25 per cent), culture 2^3 oxidized in 11 days and culture 1^4 in 18 days.
(10) Ammonium sulfate (0.5 per cent), cultures 1^7 and 2^3 oxidized in 18 days.
(11) Controls, culture 2^3 oxidized in 6 days and culture 1^4 in 9 days.

If the time required for oxidation of the sodium nitrite alone is used as a control it is plain that Nährstoff-Heyden, peptone, skimmed milk, beef extract, and beef infusion favored nitrate formation. The beneficial effect of beef extract as well as beef infusion was unexpected, since in the previous test these substances were decidedly injurious. No doubt this change in effect was due to the fact that in this experiment the nitrogenous substances were present in much smaller amounts than in the former, and were mixed with the nitrite medium which would tend to overcome any toxicity of the organic compounds. A retarding effect was noted in the oxidation of cultures containing gelatin, asparagin, urea, and ammonium sulfate.

A repetition of the preceding experiment was carried out with beef extract, 1 per cent solution, and Nährstoff-Heyden, 1 per cent solution, added in varying amounts to 10-cc. liquid cultures. Strains 1^7 and 1^3 of *nitrobacter* which had been tested for purity by inoculation into peptone-beef infusion and Nährstoff-Heyden solution, were used. For each concentration of the nitrogenous substance 3 parallel cultures were made. As might be expected, all of these cultures did not oxidize at the same time. Below are the results of this test:

Nährstoff-Heyden:

1. Culture 1^7, in the presence of 0.5, 1.0, 2.0 and 4.0 cc. of Nährstoff-Heyden solution, oxidized in 6 to 7 days.
2. Culture 1^3, in the presence of 1.0, 2.0, 4.0, 6.0 and 8.0 cc. of Nährstoff-Heyden solution, oxidized in 5 to 10 days.

Beef Extract:

3. Culture 1^7, in the presence of 0.5 cc. of beef extract, oxidized in 6 to 8 days.
   1.0 cc. of beef extract, oxidized in 9 to 26 days.
   2.0 cc. of beef extract, only one culture, out of three oxidized in 22 days.
   4.0 cc. of beef extract, no oxidation.
4. Culture 1^3, in the presence of 0.5 cc. of beef extract, oxidized in 6 to 9 days.
   1.0 cc. of beef extract, oxidized in 6 to 9 days.
   2.0 cc. of beef extract, oxidized in 8 to 11 days.
   4.0 cc. of beef extract, no oxidation.

Controls:

5. Culture 1^7 oxidized in 7 to 12 days.
6. Culture 1^3 oxidized in 7 to 16 days.

Here again the favorable effect of small amounts of nitrogenous substances, especially Nährstoff-Heyden solution, on oxidation is noticeable. Beef extract also in small amounts favors oxidation. When added in greater quantities, the beef extract retards oxidation.

SOME OF THE CHARACTERISTICS OF THE NITRATE-FORMING ORGANISM USED IN THESE EXPERIMENTS

Aside from growth in the presence of nitrogenous substances, certain characteristics of the *nitrobacter* organism were studied. Special emphasis was placed on the kind of growth on agar, the vitality of the organism, and its microscopical appearance.

Growth of nitrobacter on nitrite agar slopes

The growth of *nitrobacter* on sodium-nitrite agar slopes is very scant as compared with the growth of most organisms. It is made up of tiny beads just visible to the naked eye, more or less transparent, and of a whitish color. The culture tubes usually need to be held so that the light can come through them, in order that the faint growth along the stroke of the needle may be seen. Growth from agar slopes when inoculated into flasks of nitrite medium will cause oxidation of the nitrite comparatively fast, in a shorter time than inocula from liquid cultures.

Vitality of nitrobacter

Two strains of *nitrobacter* on agar-slope cultures which were 14 days old, were sealed and kept in the ice-box for 3 months and duplicates of these cultures were kept under the same conditions unsealed. At the end of this time, flasks of liquid nitrite medium were inoculated from them. Within 5 days one of the flasks inoculated from a sealed culture showed oxidation; in 8 days one inoculated from an unsealed tube; and in 16 days all were oxidized. At the end of a year this test was repeated and both cultures oxidized.

Three different liquid cultures of these organisms were left in the incubator at 28°C. for 30 days, at the end of which time only one drop remained of one, while two were completely dry. To see if these cultures were still alive, 10 cc. of sterilized water and a solution of 0.01 gm. of sodium nitrite were added to them. In 5 days the moist culture showed oxidation, but neither of the two which were completely dry ever gave any trace of oxidation.

Agar-slope cultures of four different strains of *nitrobacter* which had been, after the period of their incubation, kept at room temperature for 3 months and which were considerably dried, were inoculated in triplicate into liquid sodium nitrite medium. In 12 to 16 days complete oxidation had taken place in the triplicate cultures from two of the four strains, but cultures from the other two strains never gave any oxidation.

Amount of nitrate accumulated in enrichment cultures

Enrichment cultures were run of *nitrobacter* 1^7 and 2^3 to see how many additions of nitrite could be oxidized before the growth of these organisms was inhibited. The medium at the beginning contained 0.01 gm. of sodium nitrite

in 10 cc. of culture liquid. When this was oxidized, another addition of the same amount of sodium nitrite was made. In all, ten such oxidations were made covering a period of 4 months. Although sterilized water was added from time to time to make up for the evaporation in these cultures, at the end their volume was practically 5 cc. Thus the concentration was 0.123 gm. of sodium nitrate in 5 cc. of culture, or 2.46 per cent of sodium nitrate. The reaction of the cultures at this time was the same as the reaction of this medium at the beginning of the experiment, pH of 8.0 to 8.5.

Staining properties of nitrobacter

These organisms are difficult to stain and unusually so when taken from liquid cultures. The best mounts were obtained from the sodium-nitrite agar slope cultures with the use of Loeffler's flagella stain. No method of staining giving consistent results was found. Good mounts showing numerous cells were often obtained by staining freshly oxidized cultures with cold carbol fuchsin; at another time a culture of the same age and similar history would show almost no cells with this method of staining. The morphology of these organisms showed considerable variations; one of the most characteristic arrangements was that of clumps of zoöglea-like masses with only a few loose cells in the field while some mounts showed all the cells scattered more or less evenly over the field. A great many of the mounts showed the cells with rather thick straight flagella-like attachments but never more than one to a cell. Occasionally these would have the appearance of typical flagella in that they were fragile and waved. In shape the cells varied from a decided oval to almost spherical while in size equally marked differences were common.

Tubes of nitrite agar were inoculated from liquid cultures which gave no growth in bouillon or in Nährstoff-Heyden solution. After 21 days' incubation at 28°C. a faint growth could be detected. When this was stained, a small blunt bacillus, sometimes in chains, could be seen. Flasks of liquid nitrite medium were inoculated from these agar cultures and complete oxidation took place in 10 days.

Ten nitrite agar tubes were inoculated with culture 1^7 and ten with 2^8 and incubated at 28°C. Five days after inoculation a faint but noticeable growth could be seen in all the tubes. When inoculated into liquid medium all of these cultures gave oxidation, and transfers from these liquid cultures in Nährstoff-Heyden solution showed no growth. Microscopic mounts were made from the agar cultures when they were 15 days old and these were compared with mounts made from the same two cultures which were 2 months old. The organisms from the old cultures were larger and were without the flagella-like attachment, so often noted, while the 15-day cultures showed numerous polar flagella. In these mounts the flagella were twice as long as the organism and rather too thick and straight for a true flagellum. Figures 1 and 2 of plate 1, and figure 1 of plate 2 are photographs of mounts of these

cultures; and figure 2 of plate 2 is a drawing of the organisms shown in figure 1 of the same plate. The cells are unevenly stained, the center or more often one end of the cell will be well stained, while the remainder of the cell takes no stain at all except the outline of the cell wall. Often a characteristic grouping is found in these mounts similar to the illustrations.

When these cultures were a little more than 2 weeks old, they were examined microscopically again. Several good mounts were obtained. The single polar flagellum was present in all of them. Hanging-drop mounts were made from these cultures by inoculating a drop of water with growth from the agar. There was motion but it was decidedly slow. By keeping the focus carefully adjusted on one organism, it could be followed across the field; a minute or more was required for this distance.

SUMMARY

Cultures of the nitrate-forming organism were isolated from different soil types and grown on washed nitrite agar, and on slants of Nährstoff-Heyden agar with and without nitrite present. *Nitrobacter* grew on these media and retained its power of oxidizing nitrites to nitrates. Apparently the presence of a small amount of Nährstoff-Heyden was beneficial to the growth of the nitrate former. When inoculated into peptone-beef infusion or into Nährstoff-Heyden solution the pure cultures of this organism failed to show any visible growth. This statement, that no visible growth was obtained in Nährstoff-Heyden solution seemingly conflicts with the previous statement that *nitrobacter* grew on Heyden agar. Although it was not possible to determine conclusively whether growth took place in the tubes of liquid medium as shown either by its turbidity or by microscopical examinations, yet the conditions for growth in a deep layer of liquid medium compared with those on the surface of an agar slope where all the growth is concentrated in a small area, are sufficiently different to bring about results equally contradictory. Beef infusion or peptone-beef infusion, in the higher concentrations used, proved toxic for the nitrate former, whereas the Nährstoff-Heyden in nearly all tests proved non-toxic.

From examinations of microscopical preparations made from liquid cultures in water, urine, peptone-beef infusion, and Nährstoff-Heyden solution it was found that the bacteria do not reproduce in such media. Perhaps in the Nährstoff-Heyden there is a slight multiplication of the organisms.

The harmful substance found in beef infusion is present in only small amounts since dilution of the beef infusion with an equal volume of water removed this poisonous property. The nature of this harmful substance is not known, except that it is non-volatile as shown by steam distillation, and is removed by extraction with ether or alcohol.

The nitrate former will live for 2 to 6 weeks, perhaps longer, in 1 per cent solutions of Nährstoff-Heyden, gelatin, peptone, casein, yeast water, also in milk and distilled water, while in 1 per cent solutions of beef extract the bacteria are killed rapidly.

The oxidation of nitrite in cultures to which small amounts of organic nitrogen have been added takes place rapidly; in the case of Nährstoff-Heyden more rapidly than in the water controls. Such compounds as gelatin, peptone, casein, skimmed milk, beef infusion and beef extract showed no injury. Asparagin, ammonium sulfate and urea retarded oxidation.

From the results of these studies no evidence can be found to support the statements of Beijerinck that the nitrate-forming organism when grown in the presence of certain organic substances loses its power of oxidation. Contrary to much of the literature it was found that certain forms of organic matter benefit rather than injure these organisms.

Growth of *nitrobacter* on nitrite agar slants is very scant as compared with that of the common heterotrophic bacteria. The growth appears as tiny beads just visible to the naked eye. When inoculated into liquid nitrite medium, this growth brought about oxidation in a shorter time than inocula from liquid cultures.

Sealed nitrite agar slants have been kept for more than a year without serious injury to their power of oxidation.

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PLATE 1

Fig. 1. Mount made from culture 2 months old.
Fig. 2. Mount made from culture 15 days old.

FIG. 1

FIG. 2

PLATE 2

FIG. 1. Organisms from 15-day-old culture.
FIG. 2. Drawing of organisms shown in figure 1.

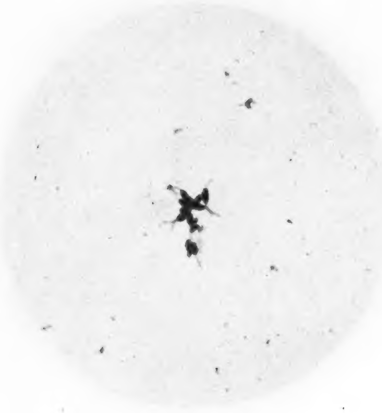

FIG. 1

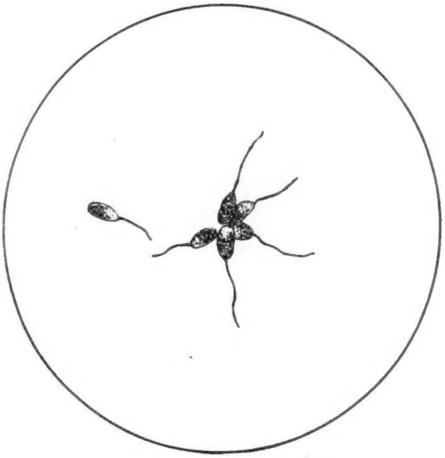

FIG. 2